DEC 14 1931

VOLUME 1 JULY, 1931 NUMBER 4

# American Journal of Clinical Pathology

OFFICIAL PUBLICATION
THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

Medical Lib.

## CONTENTS

Pathological Anatomy as the Keystone of the Practice of Clinical Pathology. KENNETH M. LYNCH ..... 277
A Comparison of Different Culture Methods for the Isolation and Growth of Mycobacterium Tuberculosis: An Experimental Study. WILLIAM H. FELDMAN ..... 285
A Comparison of Culture and Animal Inoculation of Sputum in the Diagnosis of Tuberculosis. ASYA STADNICHENKO AND H. C. SWEANY ..... 303
Cholesterol Equilibrium in the Light of Some Recent Studies. ARTHUR T. BRICE ..... 315
Glycerin as an Adjuvant to Bacterial Dyes. F. M. HUNTOON ..... 317
Pathogenesis of Goitre. B. MARKOWITZ ..... 321
Antirabic Vaccinations at the Charity Hospital of New Orleans for the Years 1929–1930. RIGNEY D'AUNOY AND J. L. BEVAN ..... 333
Degenerative Lesions of the Appendix (Appendicosis) Hitherto Undifferentiated from Appendicitis. BERNHARD STEINBERG ..... 339
Which Test for Syphilis? A. H. SANFORD ..... 347
News and Notices ..... 349
Book Reviews ..... 353

PUBLISHED BI-MONTHLY BY THE WILLIAMS & WILKINS COMPANY
MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, U. S. A.
*Made in United States of America*

# American Journal of Clinical Pathology

EDITOR

T. B. MAGATH, Mayo Clinic, Rochester, Minnesota

ADVISORY EDITORIAL BOARD

C. S. BUTLER, U. S. Naval Medical School, Washington, D. C.

H. J. CORPER, National Jewish Hospital, Denver, Colo.

B. C. CROWELL, American College of Surgeons, Chicago, Ill.

HERBERT FOX, Pepper Laboratory of Clinical Medicine, University of Pennsylvania, Phila., Pa.

A. S. GIORDANO, 604 North Main Street, South Bend, Indiana.

F. W. HARTMAN, Henry Ford Hospital, Detroit, Mich.

R. A. KEILTY, U. S. Veterans' Bureau, Washington, D. C.

R. A. KILDUFFE, Atlantic City Hospital, Atlantic City, N. J.

J. A. KOLMER, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.

K. M. LYNCH, Medical College of the State of S. C., Charleston, S. C.

S. P. REIMANN, Lankenau Hospital, Phila., Pa.

A. H. SANFORD, Mayo Clinic, Rochester, Minnesota.

W. S. THOMAS, Clifton Springs Sanatorium, Clifton Springs, N. Y.

# American Society of Clinical Pathologists

OFFICERS

*President*, H. J. CORPER, Denver, Colorado.

*Vice-President*, C. J. BUCHER, Philadelphia, Pennsylvania.

*President-elect*, W. M. SIMPSON, Dayton, Ohio.

*Secretary-Treasurer*, A. S. GIORDANO, South Bend, Indiana.

STANDING COMMITTEES

EXECUTIVE COMMITTEE

F. W. HARTMAN Detroit, Michigan.

J. H. BLACK, Dallas, Texas.

F. E. SONDERN, New York City.

C. E. RODERICK, Battle Creek, Michigan.

K. M. LYNCH, Charleston, South Carolina.

A. G. FOORD, Pasadena, California.

BOARD OF CENSORS

W. T. VAUGHAN, Richmond, Virginia.

F. H. LAMB, Davenport, Iowa.

W. E. KING, Detroit, Mich.

C. W. MAYNARD, Pueblo, Colorado.

NATHAN ROSENTHAL, New York City.

F. M. JOHNS, New Orleans, Louisiana.

BOARD OF REGISTRY

PHILIP HILLKOWITZ, Denver, Colorado.

KANO IKEDA, St. Paul, Minnesota.

M. W. LYON, South Bend, Indiana.

R. W. HAMMACK, Los Angeles, California.

A. S. GIORDANO, South Bend, Indiana.

W. E. KING, Detroit, Mich.

A. YAGUDA, Newark, N. J.


Entered as second-class matter, January 7, 1931, at the Post-Office, at Baltimore, Md., under the act of March 3, 1879

Copyright 1931, The Williams & Wilkins Company

# PATHOLOGICAL ANATOMY AS THE KEYSTONE OF THE PRACTICE OF CLINICAL PATHOLOGY*

KENNETH M. LYNCH

*Charleston, South Carolina*

The ultimate success of a professional specialty is contingent upon the attainment of "eminence in the performance of function." This I take to be the primary interest of the members of this organization.

The speciality represented here is a product of the heaping of tests and procedures to be used in diagnosis upon a profession largely untrained to make use of them. If, or when, the time may come that other practitioners may be competent, either by themselves or by means of purely technical assistance, to perform the laboratory procedures applicable to their own practice there will be no further need of the specialty.

In an estimate of the field of medical practice encompassed by clinical pathology there seemed to be three reasons for the need of recognized specialty at the present time. The first is that a large part of the practitioners of the present are untrained in the performance of methods of laboratory diagnosis; the second, that most if not all are unable to correctly interpret the applicability and the results of such tests; and the third is that a part of such laboratory diagnosis may be of such a nature as to demand such special training and experience as to create within itself a legitimate specialty.

The first of these reasons results from the fact that laboratory diagnosis has grown into medicine in mushroom fashion within a comparatively few recent years. Consequently probably the majority of physicians still in active practice were never schooled

* Presidential Address, read before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

in most of the methods now subject to every day general use. Graduates in medicine of the present time, however, are well schooled in laboratory medicine in general and as internes usually obtain sufficient experience to be reasonably competent in most of the methods which they will use in practice. Is it likely, and is it economically sound that they should abandon the benefits of this training when they enter practice? Is it necessary that they should turn it over to a specialist?

If any practitioner does his own laboratory work as well as a clinical pathologist, or if it is as practically serviceable, there is no reason why he should not and it will be economically and generally to his advantage to do so. Consequently we may expect to see and, I believe, are now seeing a tendency in the younger generation of laboratory trained physicians to make their own laboratory examinations. As their practice becomes more pressing so that they may profitably do so they may and will, no doubt, obtain the services of technical assistants, commonly in association with their colleagues.

The clinical pathologist makes much of the second reason as a justification for the specialty, that is the inability of other practitioners to interpret and make full use of laboratory examinations. This is also because a large part of the profession is unschooled in laboratory medicine. This condition, like the first, may not be one of long life. In fact it is less likely to hold with the newer generation than is that of incompetency in the actual performance of laboratory measures. The newer generation is well schooled, and will continue to be, in the interpretation of the usefulness and meaning of the various tests and procedures available. Sanford[1] thinks that the clinical pathologist will be the connecting link between the pure research laboratory and the application of its discoveries and inventions in the practice of medicine, that he will keep before the other practitioners the newer advances and interpret them in terms of actual usage. Perhaps so, but one may be skeptical that modern internists are going to be far behind clinical pathologists in knowledge of and

[1] SANFORD, A. H. The rôle of the clinical pathologist. Jour. Am. Med. Assn., **95**: 1465–1467. 1930.

practical interpretation of the available measures and methods which come from the medical research mill.

If other practitioners are going to wait supinely to be so informed there will be need of such a go-between, but a specialty built upon the shortcomings of others can live only so long as those deficiencies are not corrected.

It seems probable that other practitioners will be apt to undertake less the actual laboratory labor than the interpretation of its usefulness and meaning. Well educated physicians can just as well interpret laboratory examinations as clinical pathologists, it is conceivable that they may even believe that their own interpretations would be better in their own clientele. They can, also, do or cause to have done by supervised technicians the actual examinations in the clinical laboratory investigations now in use or apparently likely to be used. On these two counts, therefore, and if this constitutes the whole case of laboratory medicine, the permanency of clinical pathology as a definite medical specialty may be seriously questioned.

This makes the third reason the one of primary consideration. Is there a part or are there things in the actual doing of laboratory examinations which within themselves necessitate a complete medical training and expertness beyond that to be expected of other practitioners?

It needs only that one make a scanning review of the content of a text or manual on clinical laboratory examinations to be convinced that there is nothing necessarily included which may not be done by a properly and adequately trained person without any general knowledge of medicine. In so far as the performance of the actual tests and procedures and the setting down of the results goes it needs only that the doer shall be competent in an honest and reliable technique. As a matter of fact beyond the obtaining of some of the materials to be examined and the actual interpretation of the results in terms of diagnosis the strict field of clinical pathology as usually set down may not constitute the practice of medicine. In so far as an individual may make an examination for tubercle bacilli in the sputum, for albumin in the urine, or for an organism in the stool, these acts and their results

are complete within themselves. They require no general medical training and so long as the examiner assumes no responsibility in the withdrawing of materials from or other handling of the person of a patient or in the making of a dignosis to and for the patient, and so long as he or she does not enter into the treatment of the case, these things are not the practice of medicine and the laboratory examiner is responsible to no one in the matter except the physician in whose charge the patient remains.

If, however, any act concerned in or related to the performance of laboratory examinations constitutes the practice of medicine, the performer must be subject to the same considerations as other physicians in the matter of education and licensure.

In the practice of clinical pathology as now done there are numerous acts which do constitute the practice of medicine and for which the examiner is directly responsible to the patient and the state. The withdrawing of blood, body fluids, secretions, excretions, etc.; the injection or other administration of materials for functional studies; the taking of material for bacteriological studies; the performance of metabolic determinations; the injection of vaccines; the application of allergic tests; these and other quasi-clinical measures in diagnosis and in therapy come within the realm of medical practice and make it necessary that the medical laboratorian who does them shall be a licensed physician.

So long as the work comes within the field of practice it constitutes a medical specialty but it may be that it is so constituted now as a matter of practical convenience to the profession on account of conditions which may be expected to change.

These features of actual practice of medicine may, perhaps, be done by the clinician in the case, along with the interpretation of the results of such examinations, thus retaining the whole medical situation in his hands and leaving the medical laboratorian as a detached technician.

Individually considered it would seem that there is none of the practices in medical laboratories, as done at the present, which may not be handled completely by the clinician. If this is the case they should be handled by him, in considerations of efficiency and economy.

While this seems to be logical when these practices are considered individually or piece-meal it is their collective volume which makes it difficult for clinicians to encompass even the field which may be concerned in their own personal business and which makes of clinical pathology to no inconsiderable extent a specialty not of necessity but of convenience.

The gathering together of all the facilities and qualified personnel for a complete medical laboratory is generally beyond the opportunity of individual physicians, but may readily be accomplished by physicians acting in concert. As a matter of fact we now see this occurring in coöperative groups of practitioners of many varieties.

The increase of hospitalization of sick people has an important bearing here. In hospitals the laboratory facilities for a community or a certain clientele may be concentrated and it is an increasing custom to send patients into hospitals for diagnostic study on account of the availability of such facilities there. In hospital laboratories certain classifying agencies now demand that the work be under the direction of specialists of certain qualifications. In private medical laboratories which are recognized by these agencies there is a like requirement. In fact this specification of a special training in addition to a general medical education is the main pillar upon which the structure of the specialty now stands.

There is one part of the practice of pathology as a whole which probably clinical pathologists might well take into more serious consideration in guiding the future of this as an enduring medical specialty.

We have had for the duration of modern medicine the specialist known as the pathologist. Until recent times he has been mainly a teacher of general pathology in medical schools. In the practice of medicine of the present he has had an offshoot known as the surgical pathologist, whose business it is to make diagnoses from the study of diseased tissue and whose particular usefulness lies in the diagnosis of tumors, the differentiation of tumors from other pathological states, and the interpretation of the qualities of tumors by their characteristics. Along with such pathological diagnostic and prognostic studies and the routine post-operative

judgment of surgically removed tissues of all kinds, it has come into prominent recognition that the development of autopsy services as a check upon and a guide to clinical work and as a postgraduate educational measure for hospital staffs, is a major function of the hospital pathologist.

Now no one may question the necessity for a thorough general medical education and an extensive well tutored experience in this work in the qualification of a pathologist worthy of the name. There can be no question that here is constituted a medical specialty as exacting in its demands of expert medical knowledge as any other division of medicine; and it is probably a correct interpretation that the unquestionable necessities in this case rather than of those concerned with so-called clinical laboratory work are responsible for the rigid requirements set down for the hospital pathologist and the practicing pathologist in the private laboratory.

There exists at the present some distinction between a pathologist and a clinical pathologist, in that the former professes to be competent in general pathology and tissue diagnosis while the latter carries in common conception mainly proficiency in clinical laboratory work.

While in medical schools and large hospitals, particularly teaching centers, with the degree of specialization within specialties necessary to the organization of such institutions, there will, no doubt, continue to be specialists in general pathology, surgical pathology (and even subdivisions of that at times), and in clinical laboratory diagnosis, in general practice it would seem that logically the practicing pathologist should cover all of these branches.

Particularly would this seem to be the case in pathology services of hospitals, and that appears to be the purpose of the profession. It is doubtful that, even in the extent of specialization which is the fashion of the day and appears to be the trend toward the future, there is need or will be room for separation of this field into pathology and clinical pathology. It is for the purpose of arguing this matter, as of vital consideration in looking into the future of the interests of this membership, that these

questions are here presented and their evaluation attempted. It is of foremost importance that we shall analyze our own qualifications and merits, that we shall guide our own destiny to the credit of our specialty and not blindly pursue a path of present opportunity and convenience to the eventual dissatisfaction of ourselves and of the profession who now trust to us the development of the field of practical laboratory medicine.

To properly prepare oneself to practise pathology as a whole is an accomplishment of no mean proportions and of no little consumption of time. There may be those who will deny me the right to say that preparation for competency in tissue diagnosis and in judgment of morbid anatomy so far exceeds that for clinical laboratory diagnosis that the latter may be relegated to a comparatively unimportant second. That is a deliberated judgment resulting from twenty years experience, including the teaching of general pathology, the direction of a teaching hospital division of pathology, and the private practice of clinical pathology. I would go so far as to estimate that six years in the former is productive of less competency than six months in the latter. Perhaps that is the reason for the separation of pathology and clinical pathology, as conceived at the present time by the profession at large.

This is no disparagment of the qualifications of the membership of this Society of high ideals and objectives. It is our purpose to so guide the practice of pathology that it shall occupy its rightful place in the profession. In order that it may do so let us not be misguided into the line of least resistance for immediate purposes but let us logically take into account the conditions as they are now and as they will reasonably develop. Let us recognize that here we have a specialty subject to absolute control within the profession itself and, as long as it does not encroach actually upon clinical medicine, free of the commercial advantages which clinical medicine has. It does not involve pleasing the uninformed patient, it only involves satisfying the informed profession. In satisfying the profession now and in the future the practicing pathologist will find that proficiency in the science of morbid anatomy is the keystone of the structure of his specialty and that with this as the foundation the wide field of clinical laboratory

diagnosis will no doubt be left in his hands. The clinical pathologist may probably come to mean the practicing pathologist, mainly as a hospital functionary, as distinguished from one who teaches pathology.

# A COMPARISON OF DIFFERENT CULTURE METHODS FOR THE ISOLATION AND GROWTH OF MYCOBACTERIUM TUBERCULOSIS

## An Experimental Study

WILLIAM H. FELDMAN

*Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota*

Petroff, in 1915, demonstrated the practicability of isolating and growing *Mycobacterium tuberculosis* directly from infective material, and considerable impetus has been given this phase of research in tuberculosis by the use of methods described by Corper, Corper and Uyei,[3] Sweany and Evanoff, Miraglia, and others. Since difficulties were seldom experienced in obtaining cultures of the avian form of *Mycobacterium tuberculosis* on any of the mediums described by these authors, and since the suitability of each of these cultures for the production of growth of the human and bovine forms of the organism seemed to vary within wide limits, an experiment was planned to determine, if possible, which of the mediums used was the most dependable for the demonstration of acid-fast bacilli from spontaneously infected human and bovine material.

The use of suspensions of *Mycobacterium tuberculosis* prepared from cultures was purposely avoided since the bacteria in such suspensions cannot be considered comparable in cultural demands with organisms whose growing propensities have not been enhanced by previous residence on artificial mediums. One objection to the use of spontaneously infected material in a study of this kind is the difficulty of securing comparable numbers of the organisms in each culture tube inoculated. However, if many bacteria are present and several tubes of each kind of medium are inoculated it should be possible to obtain a fairly definite idea as to the ability of the respective mediums to promote the growth of *Mycobacterium tuberculosis*.

The tuberculous character of most of the specimens secured for this study was presumed by the presence in the infective material of acid-fast bacteria morphologically indistinguishable from *Mycobacterium tuberculosis*. In specimens in which acid-fast bacteria were present on the microscopic smears the organisms varied in number from few to many. The number of organisms observed varied with the character of the material examined, being most numerous in smears prepared from sputum and less abundant in smears prepared from the bovine material. Although the sputum and human tissues and the bovine material were examined for acid-fast organisms by the preparation of direct smears the urine was examined by preparing smears from the precipitate resulting from centrifuging 15 cc. of the original specimen.

## MATERIAL USED

The material of human origin was obtained from the Section on Clinical Pathology and the Section on Surgical Pathology of The Mayo Clinic and consisted of the following: thirteen specimens of urine voided and catheterized from the bladder, ten specimens of sputum, and four specimens of tissue. The specimens of sputum and urine were received in the containers in which they were obtained from the respective patients, and the infective tissues were received in sterile vials or test tubes. By appropriate staining methods acid-fast organisms morphologically similar to *Mycobacterium tuberculosis* were observed in all but one of the twenty-seven specimens. This specimen (case 7, table 1) was obtained from an infected bursa of the elbow joint and will be considered in detail later in this report.

The bovine material was obtained through the courtesy of the Federal Meat Inspection authorities from various abattoirs in South St. Paul, Minnesota. Nineteen separate specimens representing as many different animals were obtained. The specimens which were forwarded to the laboratory packed in dry borax consisted of the following: seven bronchial lymph nodes; six mediastinal lymph nodes; one cervical lymph node; one mesenteric lymph node; one specimen of lung tissue; one of a subcutaneous lesion, and two specimens of lymph tissue of undetermined origin. Smears from all but five of the bovine lesions were positive for acid-fast bacilli.

## METHOD OF INVESTIGATION

If the material consisted of tissue it was emulsified by grinding in a sterile mortar in sterile physiologic sodium chloride solution. The fluid portion was

transferred to a test tube and the heavier particles were permitted to settle. This required from one to several hours. Material containing much mucus such as tenacious sputum was washed in a small amount of physiologic sodium chloride solution by vigorous shaking, and the heavier particles were permitted to settle as for emulsified tissues. The respective specimens of urine were used without preliminary concentration by centrifugation.

Two guinea pigs were each inoculated with 1 cc. portions of the same material used for cultural attempts. One of the guinea pigs was inoculated subcutaneously and the other intraperitoneally. Each pair was placed in a separate cage and those living at the expiration of eight weeks were killed for necropsy. The presence or absence of a tuberculous infection was determined by the recognition of characteristic gross lesions. In doubtful cases histologic sections were prepared and stained for the presence of acid-fast organisms by a method previously described.[11]

Two 1 cc. portions of each specimen were each treated with 5 per cent oxalic acid solution, as recommended by Corper and Uyei,[4] and similar amounts were treated with 3 per cent sodium hydroxide, as suggested by Petroff, and by Sweany and Evanoff. This was done for the purpose of killing or inactivating contaminants which may have been present. After centrifugation all but approximately 0.5 cc. of the supernatant fluid was discarded and with a capillary pipet the precipitate was thoroughly mixed with the remaining fluid. The suspensions treated by oxalic acid were mixed with each other as were the suspensions which had been treated with sodium hydroxide and the respective suspensions were then distributed on the surface of the various culture mediums with the aid of the capillary pipet.

From each specimen, cultures were attempted on the following mediums: crystal-violet-glycerine-water-potato medium of Corper and Uyei;[3] veal-egg-cream-milk medium of Sweany and Evanoff which was slightly modified,* and

---

* In order to overcome the difficulty of obtaining a sufficient amount of filtrate from the meat-milk mixture as described in the original method, the following modification was followed: 250 grams of freshly ground round steak was added to 100 cc. of distilled water and soaked overnight at ice box temperature. It was then autoclaved at 15 pounds for fifteen minutes. While yet warm and without filtering, a mixture as follows was made: autoclaved meat broth, 100 cc.; sterile standard cream, 50 cc.; sterile whole milk, 50 cc.; eggs (whites and yolks), twice the total of the meat-broth-cream-milk mixture, or 400 cc. After being thoroughly stirred and strained through four layers of sterile gauze the medium was tubed and sterilized according to the procedure given in the original method except that the sterilization process was continued for four days instead of three.

the glycerine-broth-egg yolk medium of Miraglia.* From each of the specimens of human origin six tubes of each kind of medium were inoculated with material treated with oxalic acid, and a similar number were inoculated with the material which was treated with sodium hydroxide. With the material of bovine origin eight tubes of each kind of medium were inoculated with the material which had been treated with oxalic acid, and eight tubes were inoculated with the material treated with the sodium hydroxide. The tubes were incubated at 37°C. and were examined after the lapse of one week and twice weekly thereafter.

## RESULTS

A summary of the more important results obtained from the work with the twenty-seven specimens obtained from human sources is recorded in table 1.

From the foregoing data it is evident that a positive diagnosis of tuberculous infection was made in 24 or 88.8 per cent of the cases by the presence of lesions of tuberculosis in the inoculated guinea pig. By cultural means acid-fast organisms morphologically similar to *Mycobacterium tuberculosis* were present in twenty-three (85 per cent) of the cases. Each of the three cases which were negative by guinea pig inoculation were also negative by the cultural procedures although the culture tubes were observed for sixty-seven, eighty-seven, and seventy-two days, respectively, before being discarded. On the other hand, material from one of the cases in which acid-fast bacilli were not isolated by cultural means induced unmistakable lesions of tuberculosis in one of the inoculated guinea pigs. The material in this case (case 7, table 1)

* The medium of Miraglia was prepared as follows: seven fresh eggs were washed and immersed for ten minutes in 80 per cent alcohol; the yolks only of the seven eggs were added to 100 cc. of 6 per cent glycerine broth (the original method calls for 5 per cent glycerine); the ingredients were thoroughly mixed with an egg beater and tubed; the mixture was sterilized in the Arnold sterilizer or the insipissator, first day at 75°C. until solidified, then 85°C. for one hour, and on second, third and fourth days for 75°C. for one hour; it was incubated for two days at 37°C. before using. The glycerine broth was made as follows: Meat extract, 3 grams; peptone, 10 grams; glycerine, 48 cc., and distilled water sufficient to make 1,000 cc. were mixed. The reaction should not be alkaline and it is usually sufficiently acid without further adjustment. The broth was boiled for fifteen minutes, cooled, and filtered through paper. Distilled water was then added to restore the original volume and the broth was sterilized in the autoclave for fifteen minutes at 15 pounds pressure.

consisted of a portion of the infected bursa of the elbow joint of an adult human being. A direct smear prepared from the diseased tissue failed to disclose acid-fast organisms. Since it was thought possible that the infection might represent a bovine

TABLE 1

CHARACTER OF INFECTIOUS MATERIAL OF HUMAN ORIGIN AND RESULTS OF CULTURAL ATTEMPTS TO ISOLATE AND GROW ACID-FAST BACILLI

| NUMBER | MATERIAL | DIRECT SMEAR | RESULT OF GUINEA-PIG INOCULATION | CULTURE | RESULTS ON RESPECTIVE MEDIUMS | | |
|---|---|---|---|---|---|---|---|
| | | | | | Corper and Uyei | Modified Sweany-Evanoff | Miralgia |
| | | | | | *days* | *days* | *days* |
| 1 | Kidney | + | + | + | + 25 | + 25 C | + 25 |
| 2 | Urine | + | + | + | 15 C | + **64** | 15 C |
| 3 | Urine | + | + | + | + 35 | + 49 C | + **19 C** |
| 4 | Urine | + | + | + | + 28 | + 77 C | + **14** |
| 5 | Urine | + | + | + | + 28 | + 35 | + **18** |
| 6 | Urine | + | + | + | − 60 | + **36 C** | Not used |
| 7 | Bursa | − | + | − | − 88 | Not used | − 88 |
| 8 | Kidney | + | + | + | + 13 | + 13 | + 13 |
| 9 | Sputum | + | + | + | + 21 | + 24 | + **17** |
| 10 | Sputum | + | + | + | + 34 | + 27 | + **16** |
| 11 | Sputum | + | + | + | + 21 | + 21 | + **14** |
| 12 | Urine | + | − | − | − 67 | − 67 | − 67 |
| 13 | Sputum | + | + | + | − 72 | + 31 | + **21** |
| 14 | Sputum | + | + | + | + 30 | + **20** | + 34 |
| 15 | Sputum | + | + | + | + 17 | + 17 | + **10** |
| 16 | Sputum | + | + | + | + 22 | + 26 | + **15** |
| 17 | Sputum | + | + | + | + 20 | + 16 | + 16 |
| 18 | Sputum | + | + | + | + 27 | + 20 | + **16** |
| 19 | Sputum | + | + | + | + 26 | + 19 | + **15** |
| 20 | Urine | + | Failure* | − | − 87 | − 87 | − 87 |
| 21 | Urine | + | + | + | + 59 | + 59 | + **46** |
| 22 | Urine | + | + | + | + 30 | + 30 | + 45 |
| 23 | Kidney | + | + | + | + 24 | + **13** | + 24 |
| 24 | Urine | + | + | + | + **32** | 11 C | 11 C |
| 25 | Urine | + | + | + | 13 C | + **56** | 13 C |
| 26 | Urine | + | − | − | − 72 | − 72 | − 72 |
| 27 | Urine | + | + | + | − 65 | + 45 | + **31** |

* Died within three weeks after injection. Lesions of tuberculosis were not present.

C = Contaminants present in all tubes receiving the inoculum treated by oxalic acid and those receiving the material treated by sodium hydroxide.

Note: Bold-face type shows instances in which growth first appeared.

form of tuberculous infection, rabbits were inoculated intravenously with portions of the same material used in the guinea pig inoculations and the cultural attempts. The rabbits were without discernible lesions of tuberculosis after a lapse of sixty days. The presence of lesions in one of the inoculated guinea pigs seemed sufficient to consider the infection in this instance as due to the human type of *Mycobacterium tuberculosis*. There were three cases in which data were negative by both animal inoculation and cultural means. In only one case was there failure as a consequence of the guinea pig inoculations due to the early death of both of the inoculated animals (case 20, table 1). In very few instances was there sufficient contamination on the culture mediums to attribute to this cause the subsequent failure to obtain colonies of acid-fast bacilli. It is true that not infrequently contaminating bacteria occupied the same medium as colonies of Mycobacterium, but the latter usually were sufficiently characteristic to make their recognition easy. Often the contaminating bacteria did not become visible until the colonies of acid-fast bacilli were well established.

Comparing the efficiency of the respective culture mediums used to promote the growth of acid-fast bacilli from the material of human origin, it is apparent that the medium of Miraglia proved superior to the others in promoting the early growth of these microörganisms. In thirteen of the cases growth appeared first on the medium of Miraglia whereas in five cases growth was first observed on the modified medium of Sweany and Evanoff. Material from one specimen yielded colonies of acid-fast bacilli which appeared simultaneously on the medium of Miraglia and the medium of Sweany and Evanoff modified as described. Colonies first appeared on the medium of Corper and Uyei in only one instance (case 24, table 1) although in two of the specimens (cases 1 and 8, table 1), growth appeared on all three of the mediums simultaneously and in one instance colonies were first observed on the same date on both the crystal-violet-glycerine-water-potato medium of Corper and Uyei and on the modified medium of Sweany and Evanoff (case 22, table 1).

With the medium of Corper and Uyei acid-fast cultures were

not obtained from four of the specimens which were proved to be tuberculous by the inoculation of guinea pigs. In two other instances the medium of Corper and Uyei was so badly overgrown with contaminants after thirteen and fifteen days, respectively, that any subsequent growth of acid-fast bacilli was precluded.

On the modified medium of Sweany and Evanoff there was but one failure to obtain a growth of acid-fast bacilli from portions of the same material which produced lesions of tuberculosis in guinea pigs and in this instance the surface of all the culture mediums was completely overgrown with contaminating microorganisms after eleven days of incubation.

With the exception of three failures due to the growth of contaminating bacteria the medium of Miraglia failed to yield colonies of acid-fast organisms in only one instance in which the tuberculous character of the inoculum was proved by lesions in an inoculated guinea pig.

Although the earliest colonies occurred in most instances on the medium of Miraglia the largest number of specimens found to contain acid-fast bacilli was revealed by the modified medium of Sweany and Evanoff. Of the twenty-four specimens which were finally adjudged tuberculous as a consequence of guinea-pig inoculations twenty-two yielded acid-fast bacilli on the modified medium of Sweany and Evanoff, whereas the medium of Miraglia promoted growth from nineteen and that of Corper and Uyei's potato medium from eighteen.

In those tubes in which a growth of acid-fast bacilli occurred the growth on the modified medium of Sweany and Evanoff was perhaps the least luxuriant. Generally speaking, the bacterial growth was most abundant on the medium of Miraglia although growths of comparable luxuriance resulted in many instances on the medium of Corper and Uyei.

Most of the colonies which were examined microscopically consisted of pure cultures of acid-fast bacillary forms although in a few instances spores and yeast-like forms were observed in addition to the acid-fast bacilli. The pathogenicity of the acid-fast cultures isolated from the respective specimens obtained

from human sources was not demonstrated by animal inoculations. Therefore the designation of these organisms as *Mycobacterium tuberculosis* is entirely presumptive. More work on this phase of the problem is contemplated.

TABLE 2

CHARACTER OF INFECTIOUS MATERIAL OF BOVINE ORIGIN AND RESULTS OF CULTURAL ATTEMPTS TO ISOLATE AND GROW ACID-FAST BACILLI

| NUMBER | MATERIAL | DIRECT SMEAR | RESULT OF GUINEA-PIG INOCULATION | CULTURE | RESULTS ON RESPECTIVE MEDIUMS | | |
|---|---|---|---|---|---|---|---|
| | | | | | Corper and Uyei | Modified Sweany-Evanoff | Miraglia |
| | | | | | *days* | *days* | *days* |
| 1 | Bronchial lymph node | + | + | + | + 31 | + **27** | + 50 |
| 2 | Mediastinal lymph node | + | + | + | + 78 | + **26** | + 42 |
| 3 | Bronchial lymph node | + | + | + | − 119 | + **14** | − 119 |
| 4 | Bronchial lymph node | + | + | + | + 14 | + 14 | + 14 |
| 5 | Lung | − | + | + | + 84 | + **26** | + 41 |
| 6 | Mediastinal lymph node | + | + | + | − 87 | + 21 | + 21 |
| 7 | Subcutaneous lesion | − | − | − | − 110 | − 110 | − 110 |
| 8 | Lymph tissue | − | + | − | − 105 | − 105 | − 105 |
| 9 | Lymph tissue | + | + | + | + 49 | + **43** | − 105 |
| 10 | Mediastinal lymph node | + | + | + | + 85 | + **36** | − 90 |
| 11 | Mediastinal lymph node | − | + | + | − 90 | + **76** | − 90 |
| 12 | Mediastinal lymph node | + | + | + | − 95 | + **48** | + 82 |
| 13 | Bronchial lymph node | + | + | + | + 50 | + **44** | − 71 |
| 14 | Mesenteric lymph node | − | + | + | + 85 | + **24** | − 85 |
| 15 | Cervical lymph node | + | + | + | + 85 | + **51** | − 85 |
| 16 | Mediastinal lymph node | + | + | + | + 84 | + **30** | − 84 |
| 17 | Bronchial lymph node | + | + | Failure* | 21 C† | 21 C* | 21 C* |
| 18 | Bronchial lymph node | + | + | + | − 83 | + **49** | − 83 |
| 19 | Bronchial lymph node | + | + | + | + 76 | + **36** | + 57 |

* The culture became overgrown with contaminating bacteria before the elapse of sufficient time for possible colonies of Mycobacterium to appear.

†C = Contaminants present in all tubes receiving the inoculum treated by oxalic acid and those receiving the material treated by sodium hydroxide.

Note: Bold-face type shows instances in which growth first appeared.

In the series of nineteen specimens (table 2) obtained from bovine sources a definite diagnosis of tuberculosis was made as a consequence of guinea-pig inoculation in eighteen, approximately

95 per cent. By cultural means colonies of acid-fast bacilli were obtained from sixteen of the nineteen specimens, approximately 84 per cent. The bovine specimen (case 7, table 2) which was negative following the inoculation of guinea pigs remained negative by cultural means although the observations were continued for one hundred ten days before the culture tubes were discarded. The failure to demonstrate the presence of a tuberculous infection in this specimen should not detract from the reliability of either of the procedures used since this case belonged to a group of subcutaneous infections known as "skin lesions" which are not infrequently found in cattle which react to tuberculin. The attempt to incite lesions of tuberculosis in inoculated guinea pigs or to demonstrate the presence of *Mycobacterium tuberculosis* by cultural procedure has usually been unsuccessful and consequently the tuberculous character of lesions similar to this has not been established.

In the bovine specimens from which acid-fast bacilli were eventually isolated, the colonies appeared first on the modified medium of Sweany and Evanoff in every instance except two. In one instance (case 4, table 2) growth appeared on all mediums simultaneously, whereas in the other, colonies appeared at the same time on the modified medium of Sweany and Evanoff and the medium of Miraglia (case 6, table 2). In eleven of the nineteen attempts at growth, acid-fast bacilli were observed in the potato medium of Corper and Uyei.

In case 5 (table 2) a tube of potato medium which had been inoculated with material previously treated with 3 per cent sodium hydroxide solution developed after eighteen days a yellowish, somewhat viscid colony approximately 0.2 cm. in diameter. This colony was found to consist of acid-fast bacilli which morphologically were significantly like *Mycobacterium tuberculosis*. Pathogenicity could not be demonstrated, however, for guinea pigs, rabbits, or chickens. The original colony eventually lost its yellow color and became a dirty gray, and attempts to obtain transfers on fresh mediums failed.

Miraglia's medium promoted the growth of acid-fast bacteria in only seven of the nineteen bovine cases. Two of the seven

positive results which were obtained with Miraglia's medium represented different cases than those which were positive with the medium of Corper and Uyei.

In only one instance did the bovine specimens yield an overgrowth of contaminating bacteria which might be considered as having occurred too soon to permit the eventual growth of acid-fast bacilli (case 17, table 2). The failure of any of the mediums to promote growth of acid-fast bacteria (case 8, table 2) while the tuberculous character of the material was proved by the development of lesions following guinea pig inoculations constitutes evidence to substantiate the contention that the inoculation of guinea pigs is a more reliable procedure in the demonstration of certain tuberculous infections than cultural means.

In the experiments with the bovine material the tubes which were finally adjudged as negative were kept under observation for at least twelve weeks and a few were retained for as long as one hundred five to one hundred nineteen days before being discarded.

As in the case of the cultures obtained from the material of human source, animals were not inoculated with each of the acid-fast strains isolated from the bovine material and consequently their capacity to induce tuberculosis in susceptible animals was not proved. Further work on this phase of the problem is in progress.

The early appearance of colonies of acid-fast bacilli on all mediums in case 4 incited some doubt as to the true character of the organisms. Tests of pathogenicity subsequently proved the organism to be *Mycobacterium tuberculosis* of bovine origin since it was found to be markedly pathogenic for guinea pigs and rabbits but not for chickens.

A comparison of the results from the use of 5 per cent oxalic acid and 3 per cent sodium hydroxide for the control of contaminants failed to reveal any outstanding or consistent difference. In most of the specimens each of the reagents proved satisfactory in the elimination of nonacid-fast bacilli. It was also observed that occasionally material was encountered in which neither of these was successful in preventing the development of contami-

nants. Apparently if the contaminating organisms are excessive or if of a type which is particularly resistant to oxalic acid and sodium hydroxide in the concentrations used, neither of the reagents will effectively eliminate them from subsequent cultures.

As to the possible deterrent effect of these reagents on *Mycobacterium tuberculosis* these data fail to disclose anything of significance. In one instance (a human specimen, case 8, table 1) all mediums which were seeded with the material which had been treated with sodium hydroxide yielded acid-fast colonies consistently earlier by one week than the mediums seeded with the material which had been treated with the 5 per cent oxalic acid. In two other instances, however (cases 16 and 19, table 1), the tubes which were inoculated with the material previously exposed to 5 per cent oxalic acid developed acid-fast colonies before those which had received the material treated with 3 per cent sodium hydroxide. Another interesting observation occurred in case 21 (table 1) in which positive results were obtained from all mediums except the crystal-violet-glycerin-water-potato tubes which had been seeded with material treated by sodium hydroxide. Since the growths were luxuriant on all the other mediums it is hardly likely that the failure of a growth to appear in the potato tubes in which was material that had been exposed to the sodium hydroxide solution, was due to the absence of acid-fast bacilli in the particular inoculum used. It is evident that although certain minor differences may be noted in a comparative study of these two reagents, the use of either one would give satisfactory results in most of the specimens from which cultures may be attempted. If the material to be cultured is plentiful, the possibility of obtaining positive results in certain instances would probably be enhanced if both reagents were used for the elimination of contaminating bacteria.

As to the germicidal value of crystal violet, in the potato medium of Corper and Uyei there was no evidence that the presence of this dye had any appreciable effect on the development of bacteria other than *Mycobacterium tuberculosis*. It is true that few of the potato tubes developed growths of contaminating organisms but this was also true of other mediums which did not

contain crystal violet. When the contaminating organisms consisted of forms which were not killed or incapacitated by either the 5 per cent oxalic acid solution or the 3 per cent sodium hydroxide solution, they usually grew regardless of the presence of the germicidal dye. The color which the crystal violet imparts to the potato medium provides a contrasting background which enables one to observe minute colonies of bacteria much easier than is true with similar mediums not possessing the dye. This alone justifies the use of the crystal violet in preparing the potato medium.*

## COMMENT

Concerning the relative value of the three different culture mediums for the growth of the human and bovine forms of *Mycobacterium tuberculosis* the data obtained from this study seem significant. For the cultivation of the organism when it was of the human type the modified medium of Sweany and Evanoff and the medium of Miraglia gave consistently better results than the medium of Corper and Uyei. Although it is true that growth of comparable luxuriance eventually occurred in most instances on the potato medium of Corper and Uyei, the earlier appearance of the colonies on the medium of Miraglia pointed to the suitability of this medium for the cultivation of the human form of *Mycobacterium tuberculosis.*

These studies confirm the observations of Sweany and Evanoff concerning the advantages of their meat-milk-cream-egg mixture for the isolation of the bovine form of *Mycobacterium tuberculosis* direct from infective material. In addition the value of the modified Sweany and Evanoff medium for the isolation and growth of the human form of the organism of tuberculosis seems

* In my earlier use of the potato medium of Corper and Uyei, some difficulty was experienced in obtaining proper concentration of the crystal violet in the individual portions of potato. Dr. Corper kindly sent me some of the medium prepared in his laboratory and this served as a basis of comparison during subsequent preparation of the medium. Using the medium received from Dr. Corper as a standard of the proper concentration of dye as evidenced by the violet color, further difficulty was not encountered.

established. Apparently the modification used in its preparation did not seriously affect its usefulness.

Almost without exception the bacterial growth which was obtained from the bovine specimens was more abundant on the modified medium of Sweany and Evanoff than that which eventu-

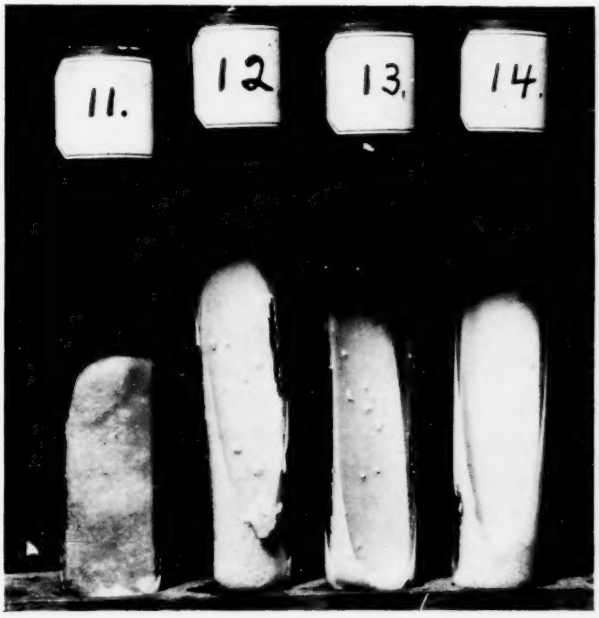


FIG. 1. RESULTS OF ATTEMPTS TO ISOLATE AND GROW THE ORGANISM OF BOVINE TUBERCULOSIS DIRECT FROM AN INFECTIVE LYMPH NODE

Tube number 11 contains the potato medium of Corper and Uyei on which growth did not occur. Tube number 12 and tube number 13 contain the modified medium of Sweany and Evanoff on which growth first appeared forty-eight days after inoculation. Tube number 14 contains Miraglia's medium on which growth did not result. The photograph was taken after one hundred four days of incubation.

ally developed on each of the others. The slowness and infrequency with which colonies of the bovine form of the organism appeared in the medium of Miraglia and of Corper and Uyei would indicate that these mediums do not qualify as satisfactory mediums for this form of *Mycobacterium tuberculosis* (fig. 1).

One can question the desirability of using either of these mediums exclusively for the demonstration of the true character of obscure tuberculous infections in human beings since the work of Aronson and Whitney and of Van Es and Martin, of Griffith and of Mitchell and of the British Royal Commission have definitely shown that the bovine type of *Mycobacterium tuberculosis* not infrequently accompanies human tuberculous infection. In all cultural attempts to demonstrate the presence of the causative organism of tuberculosis in material of human origin the use of the medium of Sweany and Evanoff, besides a medium particularly suitable for the growth of the human form, is desirable.

These data would indicate that the number of *Mycobacterium tuberculosis* present in the respective inoculums probably had a significant bearing on the lapse of time before colonies eventually appeared on the different mediums and on the luxuriance of the resultant growth. Generally speaking, there was a greater number of acid-fast bacilli in the microscopic smears prepared from the sputum and specimens of kidneys than from the specimens of urine, and the inoculums of sputum and of emulsions of kidney yielded acid-fast colonies at an earlier period than the inoculums of urine, in which the number of acid-fast bacilli was much less. This apparent relationship of the number of organisms present in the infective material to the lapse of time which passes before colonies appear on cultural mediums was also demonstrated in another study. Clinical material of human origin was used in which the presence of acid-fast organisms could not be determined by microscopic means. In every instance in which the tuberculous character of the specimen was eventually proved the appearance of the bacterial colonies occurred much later than when inoculums were observed to contain many acid-fast bacilli on examination of an appropriately stained smear.

It is obvious that more luxuriant growth will occur when many bacteria are seeded on a given medium than when only a very few are used. Consequently on suitable mediums one can expect the development of a good growth when many bacteria are present in the material to be cultured and one of less proportions when the inoculum contains a paucity of *Mycobacterium tuberculosis.*

The time which elapsed before colonies of acid-fast organisms developed from the inoculums prepared from the bovine material suggested the great variation in the biologic behavior of the bovine form of *Mycobacterium tuberculosis*. Colonies appeared as early as fourteen days (case 4, table 2) and in several instances their appearance was delayed for as long as eighty-five days. A prolonged period of incubation should be permitted before cultures inoculated with material suspected of possessing the bovine form of *Mycobacterium tuberculosis* are discarded as negative. Even with the modified medium of Sweany and Evanoff, acid-fast colonies were not discernible in one specimen (case 11 table 2) until the seventy-sixth day.

Although it is evident that cultural mediums are available which will promote the growth of most strains of *Mycobacterium tuberculosis* encountered in clinical material, these studies indicate that occasionally a strain may be encountered which stubbornly refuses to produce demonstrable evidence of growth regardless of the kind of medium used. In this connection it may be noted that whereas cultural evidence of *Mycobacterium tuberculosis* was not obtained from certain of the specimens used in this study, definite tuberculous lesions were established in guinea pigs inoculated with portions of the same inoculums from which the cultures were attempted.

Since the true character of the acid-fast organisms which were obtained in these cultural studies was not determined by tests of animal inoculation there is not available absolute proof that each strain secured was *Mycobacterium tuberculosis*. Observations lead me to believe that occasionally cultures of acid-fast organisms morphologically indistinguishable from *Mycobacterium tuberculosis* may be obtained from nontuberculous human material. Acid-fast organisms morphologically like *Mycobacterium tuberculosis* but which are not pathogenic for guinea pigs and rabbits occasionally occur in infective material of bovine origin. Such a culture was recently obtained after fifteen days incubation on an egg medium to which had been added a small amount of an aqueous extract of potato. The appearance of the colonies resembled those of *Mycobacterium tuberculosis* and a stained prep-

aration revealed a pure culture of acid-fast bacillary forms. The injection of rabbits, guinea pigs, and chickens with 1 c.c. portions of a rather heavy suspension of these organisms failed to incite the slightest evidence of tuberculosis. Although it may not be entirely valid to consider infective material of human and of bovine origin as analogous so far as the possession by each of nonpathogenic acid-fast bacilli is concerned, the possibility of bacteria of this character occurring occasionally in human material suspected of being tuberculous seems a reasonable assumption.

## CONCLUSIONS

None of the three culture mediums used proved equally efficient for the cultivation of both the human and the bovine form of *Mycobacterium tuberculosis*. For growing the organisms of human origin the modified mediums of Sweany and Evanoff and of Miraglia are considered superior, whereas for the bovine type of the organism the modified medium of Sweany and Evanoff which was slightly modified proved best. This fact would suggest the desirability of using the medium of Sweany and Evanoff in addition to any other which might be used for the isolation of *Mycobacterium tuberculosis* from the infective material of human origin in order to promote the growth of possible strains of the bovine organism which are known to occur.

Definite conclusions cannot be reached as to which of the reagents used is preferable for the elimination of contaminating bacteria. If a sufficient amount of infective material is available it is desirable that inoculums be prepared by both the oxalic acid and the sodium hydroxide method and seeded on duplicate sets of culture mediums.

## SUMMARY

A comparative study was made of the efficiency of different cultural procedures for the demonstration of *Mycobacterium tuberculosis* in infective material. A total of twenty-seven specimens of human origin and nineteen specimens of bovine origin was utilized. The culture mediums used were the crystal-violet-

glycerin-water-potato medium of Corper and Uyei; the egg-yolk-glycerin-broth medium of Miraglia and a slight modification of the meat-cream-milk-egg combination of Sweany and Evanoff. Five per cent oxalic acid solution and 3 per cent sodium hydroxide solution were used to control contaminants.

Portions of the material used for the cultural attempts were also used to inoculate guinea pigs and a slightly higher percentage of positive results were obtained from the animal inoculations than from the respective culture mediums. The modified medium of Sweany and Evanoff yielded a greater number of positive results from both the human and the bovine material. In most instances the medium of Miraglia which was inoculated with the human material promoted the growth of acid-fast bacilli earlier than the other mediums used.

## REFERENCES

(1) Aronson, J. D. and Whitney, C. E.: The types of tubercle bacilli found in tuberculous lesions and in nontuberculous tissue in man. Jour. Infect. Dis. **47:** 30–55. 1930.

(2) Corper, H. J.: The certified diagnosis of tuberculosis. Jour. Am. Med. Assn. **91:** 371–374. 1928.

(3) Corper, H. F. and Uyei, Nao: The cultivation of tubercle bacilli. An improved method for isolation from tuberculous materials. Jour. Lab. and Clin. Med. **13:** 469–480. 1928.

(4) Corper, H. J. and Uyei, Nao: Oxalic acid as a reagent for isolating tubercle bacilli and a study of the growth of acid-fast nonpathogens on different mediums with their reaction to chemical reagents. Jour. Lab. and Clin. Med. **15:** 348–369. 1930.

(5) Evanoff, Max and Sweany, H. C.: Culturing bovine tubercle bacilli. Am. Rev. Tuberc. **20:** 227–235. 1929.

(6) Feldman, W. H.: A study of subcutaneous lesions in tuberclin reacting cattle. Cornell Vet. (in press).

(7) Final report of the Royal Commission on tuberculosis. Brit. Med. Jour. **2:** 121–122. 1911.

(8) Griffith, A. S.: The types of bacilli in human bone and joint tuberculosis. Jour. Path. and Bacteriol. **31:** 875–896. 1928.

(9) Griffith, A. S.: Types of tubercle bacilli in human tuberculosis. Jour. Path. and Bacteriol. **32:** 813–840. 1929.

(10) Griffith, A. S.: The types of tubercle bacilli occurring in the sputum of phthisical persons. Jour. Path. and Bacteriol. **33:** 1145–1169. 1930.

(11) MAGATH, T. B. AND FELDMAN, W. H.: A comparison of the intracerebral method with other methods of inoculating guinea pigs for the diagnosis of tuberculosis. Am. Rev. Tuberc. **22:** 514–530. 1930.

(12) MIRAGLIA, M.: Sull'importanza della coltura del bacillo di Koch nella diagnosi della tubercolosi. Pediatria. **37:** 1167–1174. 1929.

(13) MITCHELL, A. P.: Report on the infection of children with the bovine tubercle bacillus. Brit. Med. Jour. **1:** 125–133. 1914.

(14) PETROFF, S. A.: A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. Jour. Exper. Med. **21:** 38–42. 1915.

(15) SWEANY, H. C. AND EVANOFF, MAX: Further studies in the cultivation of the tubercle bacillus. Am. Rev. Tuberc. **18:** 661–671. 1928.

(16) VAN ES, L. AND MARTIN, H. M.: The incidence of avian tuberculosis in mammals other than swine. Univ. Nebraska Agr. Exper. Station, Res. Bull. no. 49. 1930.

# A COMPARISON OF CULTURE AND ANIMAL INOCULATION OF SPUTUM IN THE DIAGNOSIS OF TUBERCULOSIS

ASYA STADNICHENKO AND H. C. SWEANY

*From the Research Laboratories of the Chicago Municipal Tuberculosis Sanitarium*

While working on our concentration method of sputum analysis we[2] encountered a number of patients in whom even by repeated concentration examinations, tubercle bacilli were never found, but roentgenologic and physical findings indicated the presence of active tuberculosis. These observations prompted us to undertake a comparative study of microscopic sputum findings with culture and guinea pig inoculations.

In this series of 200 patients, however, we did not limit ourselves to those always negative on sputum examinations with positive clinical and roentgenologic findings. We included in this series patients with minimal findings; those under observation; those with glandular tuberculosis; fibroid phthisis, and a few patients in whose sputums peculiar acid-fast micro-organisms were found. Also, we have in this series a number of patients that were positive for some time, but became negative on direct and concentration examinations, and remained so for at least nine months.

## METHOD

The specimens from these patients were usually so scant that a twenty-four-hour period was required to obtain a sufficient quantity. This factor enhanced the finding of bacilli, because we have sufficient data on hand to show that twenty-four-hour specimens give better results. So, in every instance except one, work has been done on twenty-four-hour sputum specimens. In this one exception culture and guinea pig inoculation were made directly from a hilum lymph node.

Specimens were collected in sterile glass jars, and were incubated for from sixteen to eighteen hours, in order to obtain a homogeneous specimen for accurate division. After incubation, if there was enough sputum (7 cc. or more), it was

divided into two equal parts. One was treated with acid, and the other with alkali. But if a specimen was scant, as the majority were, and only one reagent could be used, we invariably used the 3 per cent NaOH, for it gives a higher concentration. To a sputum specimen, therefore, an equal amount of 3 per cent NaOH, (or 3 per cent HCl) was added, the specimen was mixed thoroughly, and incubated for from twenty-five to thirty minutes. After incubation it was neutralized (neutral to pink and blue litmus paper), and the specimen was centrifugated for about twenty minutes. The supernatant liquid was poured off; a smear was made and examined very carefully for the presence of acid-fast bacilli. Only negative sputum specimens were used for the experimental work, with two exceptions, which will be discussed later.

The majority of the specimens came from our Sanitarium, and were, therefore, fresh. Unfortunately, this was not true about the ones that came from Dispensaries, from which deliveries could not be made to the laboratory on the same day they were collected. As a result, cultures of Dispensaries' specimens were contaminated more frequently than the others.

Cultures and guinea pig inoculations were always made on the same specimen, unless specified. The guinea pigs were killed between six weeks and two months after inoculation. The cultures were examined for the growth of tubercle bacilli twice a week for at least two months before they were discarded as negative.

## RESULTS OF STUDY

This series of 200 patients may be divided into four groups based on cultural findings and guinea pig inoculations. Out of these 200 patients, forty-nine were children less than fifteen years of age. At present we feel that this group of patients should be studied much more carefully, and guinea pig inoculation should be done not only with the sputum specimens but with feces and throat swabs at the same time.

The first or largest group in this series, consists of 131 patients (65.5 per cent). These patients were found to be negative both on culture and guinea pig inoculations. Of these 131 patients ninety-five were never found positive by any method employed in the laboratory. Thirty-six have been found positive by microscopic examinations, but became negative and remained so even on culture and guinea pig inoculations.

Table 1 gives data on ninety-five patients who were never found positive by any laboratory method.

It is obvious to anyone why most of these patients may become

negative. If there is any question, it may be raised about the negative far advanced patients. This ought to be no mystery, because these patients occasionally become entirely healed as well as those having earlier stages of the disease.

Among these ninety-five patients two deserve special consideration, for in both of these "peculiar acid-fast microorganisms were found."

(1) M. F., Racine Avenue Dispensary No. 26656, was a white male, fifty-two years old, who was first examined in 1928 and was diagnosed as far advanced 'B." Since that time until May 27, 1930, fifteen examinations of sputum were

TABLE 1

CASES NEGATIVE BY EVERY METHOD

| DIAGNOSIS | CASES | NEGATIVE EXAMINATIONS |
|---|---|---|
| For observation | 9 | 105 |
| Glandular tuberculosis | 9 | 105 |
| Minimum "A" | 9 | 114 |
| Minimum "B" | 15 | 196 |
| Moderately advanced "A" | 15 | 290 |
| Moderately advanced "B" | 21 | 323 |
| Far advanced "A" | 5 | 102 |
| Far advanced "B" | 6 | 110 |
| Abscess | 2 | 104 |
| Bronchiectasis | 3 | 70 |
| Thyrotoxicosis | 1 | 11 |
| Total | 95 | 1,530 |

made; also a guinea pig inoculation and culture were made on November 17, 1929, all of which were negative. On May 27, 1930 "peculiar clubby acid-fast bodies" were found. Another guinea pig and culture were made on June 17, 1930, but they were also negative.

It is unfortunate that in both these cases we were not able to use the specimens in which the "peculiar forms" were observed for cultures and guinea pig inoculations, and that in the specimens that were used for inoculations no acid-fast forms were found.

(2) R. P., Washington Dispensary No. 26050, was a white female who had been under supervision of the Dispensary since 1908. At that time the case

had been diagnosed as arrested moderately advanced pulmonary tuberculosis. Until 1924 she had been in good condition. In March, 1924 she developed a hemorrhage, but had no record of positive sputum, even at the time of the hemorrhage. On June 14, 1930 "peculiar acid-fast bacilli" were found. (They were very solid and thick, in some places they were finer, but not a single slender typical tubercle bacillus was found.) A twenty-four-hour specimen was obtained, and culture and a guinea pig inoculation were made, with negative results.

Table 1A presents the data on the thirty-six cases in this group. These thirty-six cases, as noted before, were found to be positive but became negative, and all of them save three were negative at least nine months before guinea pig inoculations and cultures were performed on them.

TABLE 1A

CASES ONCE POSITIVE, NOW NEGATIVE BY EVERY TEST

| DIAGNOSIS | CASES | NEGATIVE EXAMINATIONS |
|---|---|---|
| For observation | 2 | 15 |
| Minimum "A" | 9 | 118 |
| Minimum "B" | 5 | 62 |
| Moderately advanced "A" | 4 | 36 |
| Moderately advanced "B" | 4 | 52 |
| Far advanced "A" | 7 | 159 |
| Far advanced "B" | 5 | 74 |
| Total | 36 | 516 |

Among these thirty-six patients, three deserve a detailed discussion.

(1) C. R., State Street Dispensary No. 23302, white female fifteen years old. This child was under observation on account of being twenty pounds underweight. Physical findings were practically negative. No roentgenogram was made. On March 24, 1930 two bacilli per slide were found, and on April 11, 1930 another positive twenty per slide was found. (Both positives were found on concentrations.) On May 9, 1930 guinea pig inoculation and culture proved to be negative. Three other microscopic examinations, following guinea pig inoculation, were negative also.

(2) M. N., State Street Dispensary No. 23311, white, male, twenty-two years old, was under observation. On March 18, 1930 the first sputum examination was found positive twenty per slide (concentration). Eight other examinations, all concentrations, were found negative. Guinea pig inoculation and culture made April 9, 1930, with negative result.

(3) R. M., Grand Crossing Dispensary No. 27249, was a white female who had been under supervision since July, 1928. The physical findings at that time revealed a fifteen pound loss of weight, apparent anemia, clubbed fingers, impaired resonance at the right upper lobe, with râles. Twelve sputum examinations were negative. On March 5, 1930 positive sputum, fifteen per slide (concentration) was reported. On June 14, 1930 a twenty-four-hour specimen was obtained for guinea pig inoculation and culture (this specimen was found positive two per slide), but it failed to produce any lesion in the guinea pig, and the culture was negative. Two more animal inoculations and cultures were made, all with the negative results. On September 30, 1930 three specimens were sent in. Two were negative, but the third revealed two acid-fast bacilli per slide (concentration). Two more guinea pigs and a rabbit were inoculated, but results were negative also. The patient is losing weight steadily, and has a moderate cough with expectoration and moist râles in both apices. This

TABLE 2

CASES NEGATIVE MICROSCOPICALLY, BUT POSITIVE ON GUINEA PIG AND CULTURE

| DIAGNOSIS | CASES | NEGATIVE EXAMINATIONS |
|---|---|---|
| Minimum "A" | 1 | 25 |
| Minimum "B" | 1 | 18 |
| Moderately advanced "A" | 3 | 53 |
| Moderately advanced "B" | 4 | 82 |
| Far advanced "A" | 1 | 18 |
| Far advanced "B" | 3 | 56 |
| Total | 13 | 252 |

patient apparently has an open case of tuberculosis, but our inability to produce tuberculosis in animals, we fail to explain, unless it is avirulent organism, or an organism that is killed by concentration treatment.

The second group in this series has thirty-three patients (16.5 per cent). Those patients were found positive both on guinea pig inoculation and culture. Of these, thirteen were never found positive before; seventeen were found positive before by the microscopic examinations, but they have been negative at least nine months before guinea pig inoculations and culture were made on their sputum. Table 2 gives information concerning thirteen patients; and table 2a gives data on seventeen patients. Three other patients of this series will be discussed separately.

(1) F. T., Washington Dispensary No. 10094, white male, forty-eight years old, had been under supervision of the Dispensary for ten years. On physical examination he was diagnosed as far advanced. His sputum was never found positive, although thirty-one microscopic sputum examinations were made. On November 23, 1928 his sputum was found positive for the first time, fifteen per slide (concentration). A twenty-four-hour specimen was obtained and was found positive, twelve per slide. From this positive specimen guinea pig inoculation and culture were made. The culture showed food growth of acid-fast bacilli, but the guinea pig showed no evidence of tuberculosis. Another positive specimen was obtained one per field (concentration), from which an animal inoculation and culture were made. The culture produced luxuriant growth; the guinea pig developed an enlargement of spleen and inguinal lymph nodes; smear from which revealed acid-fast bacilli. The Dispensary record shows that

TABLE 2A

CASES ONCE POSITIVE, NOW NEGATIVE MICROSCOPICALLY, BUT GUINEA PIG AND CULTURE POSITIVE

| DIAGNOSIS | CASES | NEGATIVE EXAMINATIONS |
|---|---|---|
| Minimum "A" | 3 | 52 |
| Minimum "B" | 3 | 43 |
| Moderately advanced "A" | 5 | 70 |
| Moderately advanced "B" | 4 | 78 |
| Far advanced "A" | 1 | 16 |
| Far advanced "B" | 1 | 12 |
| Total | 17 | 271 |

the patient rapidly grew worse after the first positive sputum was found, and died a month after the second guinea pig was inoculated.

(2) C. B. was a white male, under a private physician's care. He was first seen in August, 1929 when he suffered from massive hemorrhages and acute bilateral pulmonary tuberculosis. His first sputum was submitted on March 13, 1930, and was found positive, four per slide (concentration). The report was held, for two other specimens sent on the same day were negative. Guinea pig inoculation and culture were made on April 9, 1930 from twenty-four-hour specimen. Both proved to be positive.

(3) M. J., M.T.S. No. 28044, a white female, forty-three years old, entered the Sanitarium in March, 1929. Sputum was examined fifteen times, all of them negative. Patient complained of cough with expectoration, streaked sputum, loss of twenty-five pounds in weight, pain in chest, shortness of breath on exertion, and loss of appetite. She had had bronchitis five months prior to this, with a slight amount of yellow sputum, and an "abscess" in the throat a year

before. After admission she gradually became worse, and developed fluid in the chest that was found negative for *mycobacterium tuberculosis* (guinea pig was inoculated). The physical examination revealed impaired resonance at the apices, with no râles. The right base contained fluid. Roentgenogram revealed some dense hilum shadows. All laboratory examinations were negative for tubercle bacilli, or any other definite findings. The patient died on January 17, 1930, and a necropsy revealed a massive involvement of the hilum lymph nodes, with extension along the right main bronchus and up to the trachea. A gross diagnosis of Hodgkin's disease was made, but microscopic section revealed tuberculosis. A guinea pig inoculation and culture that were made from the hilum lymph node revealed virulent bacilli.

The third group in this series consists also of thirty-three patients (16.5 per cent). Those patients were found positive on

TABLE 3

CASES NEGATIVE TO MICROSCOPIC SPUTUM EXAMINATION BUT POSITIVE ONLY BY GUINEA PIG INOCULATION

| DIAGNOSIS | CASES | NEGATIVE EXAMINATIONS |
|---|---|---|
| For observation | 3 | 36 |
| Glandular tuberculosis | 1 | 15 |
| Minimum "A" | 1 | 23 |
| Minimum "B" | 5 | 81 |
| Moderately advanced "A" | 4 | 79 |
| Far advanced "A" | 1 | 20 |
| Total | 22 | 341 |

guinea pig inoculation, only. Out of these thirty-three patients, twenty-two were never found positive before.

Table 3 gives information concerning those twenty-two patients.

Table 3A gives data on eleven patients who were positive by sputum examination once, but have been negative for at least nine months.

In this group, as in the two previous ones, cultures were made parallel with the guinea pig inoculations on all the patients, except one. In that one instance untreated sputum was used for a guinea pig inoculation, and therefore no culture could be made.

This patient was diagnosed as far advanced "B" with tuberculous

laryngitis. He was found positive only twice, and twelve other examinations were negative. We suspected that in his sputum acid-fast bacilli were destroyed by the treatment, so we employed untreated sputum for a guinea pig inoculation. We were successful in producing tuberculosis in a guinea pig, although previous attempt to infect animals with the treated sputum had failed.

In this group, therefore, we have thirty-two patients on whose sputum specimens cultures were made parallel to animal inoculation. Of these thirty-two cultures, ten were contaminated. During the time before cultures could be made different bacteria might multiply in such great numbers that it would be quite often

TABLE 3A

CASES ONCE POSITIVE, NOW NEGATIVE MICROSCOPICALLY AND CULTURALLY, BUT GUINEA PIG POSITIVE

| DIAGNOSIS | CASES | NEGATIVE EXAMINATIONS |
|---|---|---|
| Minimum "A" | 3 | 46 |
| Moderately advanced "A" | 1 | 6 |
| Moderately advanced "B" | 4 | 53 |
| Far advanced "A" | 1 | 14 |
| Far advanced "B" | 3 | 57 |
| Total | 11 | 176 |

impossible to kill them off with our treatment. Elimination of incubation could help in preventing rapid multiplication, and would save time, but we found incubation of a great help in digesting mucoid material in preparing a homogeneous sputum.

The fourth group consists of only three patients (1.5 per cent). These three patients were found positive by the culture method only.

(1) E. M., No. 27334, was a white male, forty-one years of age. Clinically a diagnosis of far advanced "A" was made. Eight sputum examinations were negative. The guinea pig inoculation on December 6, 1928 was negative, but the culture made from the same specimen was positive. The virulence test on the bacilli from the culture was not done.

(2) J. M. M., No. 29310, was a white male forty-nine years old. He entered

the Municipal Tuberculosis Sanitarium in September, 1929. His complaints were loss of weight and productive cough. The diagnosis was moderately advanced "A" with bronchitis. Twenty sputum examinations (10 direct smears and 10 concentrations) were negative. On April 14, 1930 a guinea pig inoculation and culture were made. The guinea pig was negative, but culture revealed growth of acid-fast bacilli, which proved to be virulent.

(3) K. D., No. 29945, white female, nineteen years old. The father and grandfather had died of tuberculosis. Her complaints were loss of weight, slight expectoration, cough, pain in both sides of the chest. There was impaired resonance in the right apex with few râles. Whispered voice was slightly increased. Since January, 1930 twenty sputum examinations were made, all negative. On June 13, 1930 a guinea pig inoculation and culture were made. The culture became contaminated in a few days, so it was repeated on June 22, 1930. This last culture produced a good size colony of acid-fast bacilli, which were found to be virulent for a guinea pig. The guinea pig that was inoculated with sputum on June 13, 1930 showed no evidence of tuberculosis.

The results obtained on this series of 200 selected patients reveals that animal inoculation is still the method of choice in the diagnosis of tuberculosis with certain types of septic material. There are two factors that interfere with accurate culture diagnoses; first, spore forming contaminations, and second, what appears to be weakly protected tubercle bacilli that seem to be killed in the treatment with chemicals. Spore forming bacilli are nearly always present in feces; occasionally in urine, and in most old samples of sputum. In such samples, therefore, it is not safe to risk a diagnosis on cultures alone. Furthermore, we have noticed on numerous occasions some specimens in which treatment has killed the tubercle bacilli, untreated specimens have given growth or infected guinea pigs. Where other bacteria are present, however, cultures are impossible without treatment. Barring these two types, culture work is superior. Its chief advantages are the low cost, and the fact that daily examinations may be made over a long period of time, increasing the chances of finding the bacilli. Furthermore, the value of a positive culture is absolute, while animals are subject to spontaneous infection that occasionally occurs.

We regret that the scant amount of sputum prevented us from using other media, besides cream, for we feel that the comparative study of the different media on this kind of specimens would be

of a real value. Also, inability to use alkali and acid treatment on all the specimens we feel was a drawback in our cultural work. We have enough evidence from our previous and present work to believe that certain specimens produced growth only when treated by acid, and others by alkali.

From a clinical standpoint, this study reveals that there are a certain percentage of early and moderately advanced cases having clinical disease that apparently never expel tubercle bacilli in the sputum; that there are others which may do so occasionally; and that still others become sputum negative from a few months to years after the beginning of treatment. The same rule also applies to far advanced patients, except the time limit extends to years and decades. Many far advanced patients have become negative only after ten or fifteen years of treatment; others become quiescent and become positive from time to time; while others (the greater number) never become negative.

## SUMMARY

A comparative study of microscopic sputum examinations, with guinea pig inoculation and culture, were made on selected series of 200 patients, forty-nine of which were children under fifteen years of age.

Twenty-four hour sputum specimens were used for the experimental work, and treated with 3 per cent NaOH. Half were inoculated into a guinea pig, and half were cultured on the cream medium of Sweany and Evanoff.[1]

This series was divided into four groups, based on cultural and animal findings.

The first, or largest group, consists of one hundred and thirty-one patients, found negative both on guinea pig inoculation and culture.

The second, consisting of thirty-three patients, were found positive both on guinea pig inoculation and culture.

The third group, consisting also of thirty-three patients, were found positive on guinea pig inoculation only.

The last, or fourth group, consisting of three patients, were found positive on culture only.

The experimental work gave us sufficient data to believe that guinea pig inoculation was superior to cultural method for badly contaminated material, but culture study has the advantage of being more absolute, more inexpensive, and permitting of more repetitions.

Atypical acid fast bacilli should be carefully considered but only as suspicious forms until they have produced typical tuberculosis in animals.

## REFERENCES

(1) SWEANY, H. C. AND EVANOFF, MAX.: The isolation of tubercle bacilli from septic material. Amer. Rev. Tuberc., **17**: 47–52. 1928.

(2) SWEANY, H. C. AND STADNICHENKO, ASYA: A review of the incubation method of sputum concentration. Amer. Rev. Tuberc., **22**: 420–442. 1930.

# CHOLESTEROL EQUILIBRIUM IN THE LIGHT OF SOME RECENT STUDIES

ARTHUR T. BRICE, JR.

*From the Laboratory of the United States Veteran's Bureau Hospital No. 24 and Diagnostic Center, Palo Alto, California*

I have recently published data on a series of 1,127 valid micropolariscopic examinations of specimens of pathologic urine.[1] These indicated an average incidence of doubly refractive lipoids in pathologic urine of all groups of about 13 per cent. The group of 725 specimens from surgical cases is of special interest. Study of these specimens in relation to involvement of the intestines showed an average positive incidence of urinary lipoids of 21 per cent when the intestines were involved, with a positive incidence of but 8 per cent where there was no involvement of the intestines, as shown in the tabulation.

Through the kindness of Dr. Wm. H. Wilmer, Dr. Warfield T. Longcope, and Dr. Dean Lewis, I have recently been able to check the interpretation of my earlier findings on two patients with gall bladder disease and seven patients with hernia operated on in the surgical clinic of the Johns Hopkins Hospital. Daily specimens of urine, from date of admission to date of discharge, were examined by the micropolariscope in each of the cases. Urinary lipoids were found in every case. The gall bladder cases showed doubly refractive urinary lipoids in 97 per cent of the valid examinations, the finding consisting principally of blastomatous granulo-cellular elements, which I have elsewhere designated lipoid crystals, and of lipoid epithelium.

In six of the seven cases of hernia there was marked displacement of the intestine. In the other case there was severe strangulation and intestinal involvement. This latter case showed presence of lipoid bearing, doubly refractive, round and caudate epithelium as well as the more common squamous lipoid bearing cells. There was a concurrent renal involvement in only one

case, and strangely enough this was the only case examined in which the typical lipoid granule showing characteristic polarizing cross-figure was not found. The specimens from the cases of hernia as a group showed presence of urinary lipoids in 61 per cent of the valid examinations. Fourteen per cent of all the positive findings consisted of the typical granule of characteristic polarizing cross-figure. Thanks are due to Dr. G. G. Finney, Resident Surgeon, for grading the extent of intestinal involvement in the individual cases.

INCIDENCE OF URINARY LIPOIDS FOUND IN 725 SPECIMENS FROM SURGICAL PATIENTS

| INTESTINES INVOLVED | | INTESTINES NOT INVOLVED | |
|---|---|---|---|
| | *per cent* | | *per cent* |
| Biliary tract surgery | 27 | Tumors (miscellaneous) | 18 |
| Salpingectomies | 24 | Minor surgery | 17 |
| Herniotomies | 20 | Thyroid surgery | 15 |
| Miscellaneous laparotomies | 18 | Genito-urinary surgery | 8 |
| Appendectomies | 17 | Gynecological surgery | 4 |
| | | Infections | 4 |
| | | Eye, ear, nose, throat surgery | 4 |
| | | Kidney surgery | 0 |
| | | Traumatic cases | 0 |
| Average incidence | 21 | Average incidence | 8 |

These findings would appear to indicate that in pathologic conditions there may be elimination of lipoids by the kidneys proportional to the extent of the intestinal involvement. They also give added credence to the work of Gardner and Gainsborough[2] who have shown that distinct changes of level of blood cholesterol, as well as changes in its proportional distribution between the free and the combined forms, which may be either up or down, do occur during digestion, and are demonstrable within three or four hours after the ingestion of meals of varying composition.

## REFERENCES

(1) BRICE, A. T., JR.: The incidence of lipoids in urine. Jour. Lab. and Clin. Med. **15**: 953–960. 1930.

(2) GARDNER, J. A., AND GAINSBOROUGH, H.: Cholesterol content of normal human plasma. Biochem. Jour., **22**: 1048–1056. 1928.

# GLYCERIN AS AN ADJUVANT TO BACTERIAL DYES

F. M. HUNTOON

*From the White Haven Sanatorium, White Haven, Pennsylvania*

In every laboratory where bacterial staining is a routine procedure and particularly in those laboratories where such staining is only an occasional necessity, annoyance has been caused by the tendency of certain prepared dye solutions to deteriorate and to precipitate either on the slide or in the bottle. This is especially annoying in the dyes used to perform the Gram stain.

In a search for dye solutions that would not have these characteristics, we hit upon the use of glycerin as an adjuvant. This substance in the proper strength acts, first, as a preservative in helping to keep the dye stuffs in solution, second, apparently as a mordant in increasing staining values, and third, aids by clearing up the background, giving very sharp and clear microscopical pictures.

## THE GRAM STAIN

### *Glycerin crystal violet gram*

The solutions are made up as follows:

Mix:
15 parts—3 per cent solution of crystal violet in 95 per cent alcohol.
85 parts—30 per cent solution of glycerin in water.

Counterstains:

A. Red counterstain. Mix 10 cc. of carbolfuchsin with 100 cc. of a 25 per cent solution of glycerin in water.

B. Brown-yellow counterstain. Shake up 2 grams of Bismark brown in 100 cc. of water and filter. To the filtrate add 30–40 cc. of glycerin and mix.

The other solutions employed are the standard Gram's iodine solution and an acetone alcohol decolorizer (75 parts alcohol to 25 parts acetone). Alcohol alone may be employed but decolorization is somewhat slower.

The technique of staining is that of the standard Gram.

1. Cover the film with the glycerin crystal violet solution for one to two minutes. Wash in water.
2. Cover the film with Gram's iodine one minute. Wash in water.
3. Decolorize with acetone alcohol a few seconds until color is practically gone. Wash in water.
4. Counterstain with either the A or B counterstain 30 seconds. Wash in water.
5. Dry and examine.

The above procedure results in microscopical preparations of great beauty, showing a very sharp differentiation against an extremely clear background. The advantages over the usual method are especially apparent in examining smears from exudates where the Bismark brown counterstain is to be preferred.

Variations of the times given are allowable without influencing the results, making it an efficient method even in inexperienced hands.

Using alcohol as a decolorizing agent we have decolorized Gram negative cocci in ten seconds and failed to decolorize *Staph. aureus* in two hours.

### TUBERCLE BACILLUS STAINS

The same staining solution (glycerin crystal violet) given above, may be used for the staining of tubercle bacilli in sputum. It is handled in the same manner as carbolfuchsin: steaming one to two minutes or longer; if accidentally boiled, the stain does not precipitate. Wash in water and decolorize by any of the standard methods, wash again, and counterstain with Bismark brown.

The tubercle bacilli appear as slender black rods against a yellow background and are apt to appear thinner and more beaded than with a carbolfuchsin stain.

Although some of those who have seen this stain prefer it in searching sputa for tubercle bacilli, the author prefers the conventional red-blue picture obtained with the carbolfuchsin-methylene blue stains. These preferences seem to be based on a question of eye strain noted where large numbers of slides are examined. One finds as many organisms with one method as the other.

*Glycerin carbolfuchsin*

We prepared a glycerin carbolfuchsin stain as follows:

Phenol, 5 per cent, 75 cc.
Glycerin, 25 cc.
Saturated solution of basic fuchsin in 95 per cent alcohol, 10 cc.

This combination stains as well as the usual carbolfuchsin, but does not tend to precipitate either in the bottle or on the slide even if boiled. It also gives clearer backgrounds.

GLYCERIN METHYLENE BLUE

A modified Loeffler's methylene blue was made as follows:

Twenty-five per cent solution of glycerin in water, 100 cc.
1 per cent solution of sodium hydrate, 1 cc.
Saturated solution of methylene blue in 95 per cent alcohol, 10 cc.

This mixture stains equally well or better than the usual Loeffler's methylene blue, contains one third the amount of dye stuff and gives sharper pictures.

An ideal blue counterstain for use with the carbolfuchsin tubercle bacillus stain is prepared by adding 2 cc. of saturated alcoholic solution of methylene blue to 100 cc. of a 25 per cent solution of glycerin in water.

The dye solutions given above all keep well, which fact is surprising in the case of the Bismark brown solution but also gratifying since it enables a routine use of this excellent dye.

SUMMARY

We have prepared and used certain modifications of the dye solutions in common employment for bacterial staining in working laboratories and have found that the addition of glycerin not only aids in the keeping properties of these solutions, but that it aids in the staining, greatly increasing the clearness of the microscopical picture.

The Gram method given has been in use in the laboratory of large general hospital for a year with consistent results. This and our own experiences justify us in offering these methods as a help to the laboratory worker.

# PATHOGENESIS OF GOITRE*

B. MARKOWITZ

*From the Pathological Department of the Sloan Clinic, Bloomington, Illinois*

Consideration of the method whereby pathologic changes occur in the thyroid gland usually results in a goitre classification which is in accordance with the conception of the mode or path the goitre has traveled. The construction of a classification which will be acceptable to both pathologist and surgeon seems rather difficult. This may be explained by the fact that the histologic findings often fail to coincide with the clinical picture. It would be a comparatively simple problem if the different types of goitre were distinct clinical entities and the observations of the pathologist coincided with those of the clinician. The large number of various classifications, however, testify to the lack of agreement as to the interpretation of goitre pathology not only between clinicians and pathologists but also between members of each group. One of the simpler classifications is the one offered by Marine[3] in which he divides the diseases of the thyroid, due to the disturbances of its function, into two groups: (1) thyroid insufficiency and (2) exophthalmic goitre. These two groups are designated hypo- and hyperthyroidism even though they very often overlap.

From the very origin of the embryonal formation of the human thyroid to the time the gland reaches maturity in adult life, we find colloid storage (supply) on the one hand and lack of colloid with hyperplasia (demand) on the other. The maintainence of a balance between these two seems to be the function of this gland. During various periods of life such as pregnancy, puberty and adolescence, the thyroid in man normally changes in texture depending upon the stage of development, and these changes are generally interpreted as due to increased or decreased activity.

* Read before the Ninth Annual Convention of the American Society of Clinical Pathologists, Detroit, Michigan, June 20–23, 1930.

With increased activity or greater demand there is, histologically, a marked decrease in colloid containing follicles, a definite increase in the number of cells making up the follicles and general increased vascularity. With decreased activity or supply, the opposite histological picture results: there is a decrease in the number of follicular cells and marked colloid storage in the follicles. The necessity of this simple hyperplasia and its associated increased activity is, most likely, a part of the body mechanism in supplying whatever internal secretion is demanded at that particular period of life and the histological findings of hyperplasia coincide with the clinical symptoms of hypertrophy and increased activity. If left alone this thyroid may revert to normal size and no further clinical symptoms of increased activity may manifest themselves when this particular period of life is passed. The demand which was evidenced by hyperplasia has been supplied and the gland reverts to the normal colloid resting state. There is reason to believe that this normal process is the basis, in its simple form, of disturbed thyroid function resulting in many various stages or so-called goitre types; that goitre production is dependent upon the extreme exaggerations of the normal capacities of the thyroid gland. Chart 1 gives a probable explanation of the route the gland travels in producing various types of goitres.

With the very beginning of thyroid dysfunction it seems probable that hyperplasia fails in its duty of properly supplying the demand and whatever the cause, hyperplasia is increased instead of the gland reverting to the normal resting state. With the ability of adjustment possessed by the animal body it is quite conceivable that the demand may finally be met with a proper supply but only after hyperplasia has progressed to a stage which is above that found in the normally functioning thyroid; and because hyperplasia has already passed the stage of normal, this reversion imitates and simulates, but never reaches the normal. It assumes the resting state as it attempts, but because the hyperplasia has exceeded its normal limits the resting state is now also abnormal in that an over-abundance of colloid has been produced and a simple colloid goitre results.

Colloid goitre is what the name indicates; an excessive collec-

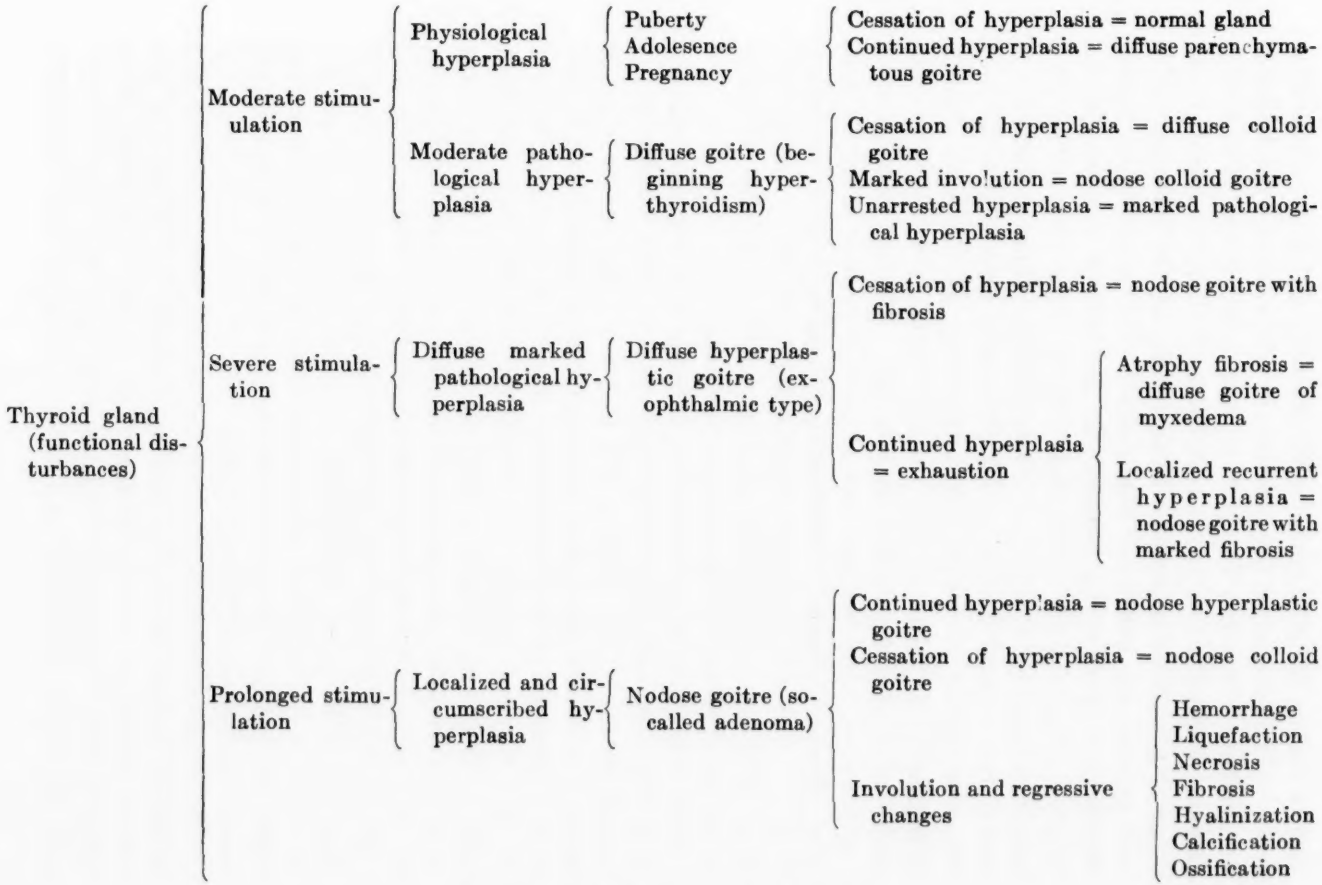


CHART 1. THE PRODUCTION OF GOITRE

tion of colloid in the follicles with thinning of the interfollicular septa. The cells lining the follicles are flattened, and the follicles are increased in size. The picture, in general, is one of rest and decreased activity but yet in certain areas there are small buddings or plications with some hypertrophy of the follicular cells and generalized increased vascularity. These are most likely remnants of preceeding hyperplasia and testify in favor of increased activity with hyperplasia having been followed by excessive amounts of colloid and storage of this colloid in the dilated and hypertrophied follicles of simple colloid gitre. Here is seen an overlapping of hypo- and hyperthyroidism; with simple hyperplasia the latter occurs, whereas during the colloid resting state (colloid goitre) the former is found. Because under certain physiological body changes and at certain periods of life similar hyperplasia is followed by the resting colloid state, it is reasonable to assume that the simple colloid and adolescent goitres are dependent upon the same process which normally operates although in somewhat greater proportions.

In nodular formation it is quite probable that a single localized area of hyperplasia failed to become arrested and to recede to the colloid resting state. Within this area new follicular structure is formed with increasing lack of orderly arrangement and failure of development of the stroma that characterizes normal thyroid tissue. Further cellular proliferation within this localized area continues, causing it to increase in size and with increasing growth more and more pressure is exerted upon the surrounding tissue. In the immediate periphery atrophic changes occur and replacement fibrosis of the portions undergoing atrophy isolates this area of irregular hyperplasia. As the process continues the the zone of replacement fibrosis becomes compressed and consolidated, completely circumscribing and finally encapsulating a mass of proliferating thyroid tissue.

The development of nodular goitres by this process of increased growth in certain areas, irregular hyperplasia and involution, isolation, complete circumscription and finally encapsulation is borne out by considerable evidence and many investigators support this view (Rienhoff and Lewis,[5] Graham[1] and Hertzler[2]).

That such a process takes place in the thyroid gland is given support by the transition from apparently normal non-nodular thyroid tissue to nodular goitre as seen in the glands of individuals approaching or who have attained adult life. This is particularly seen in females where hyperfunction of the thyroid occurs at certain periods as a physiological phenomenon.

While the colloid resting stage is here an attempt to reversion to normal it has not achieved its object completely, but has reverted to a degree as near normal as possible. Assuming this process to be the one involved, that of simple hyperplasia beginning in the normal thyroid gland which when arrested results in a simple colloid goitre, it must be admitted that there is a possible relationship between hyperplasia, colloid goitre and normal thyroid function.

The ability of bodily adjustment may however fail in some instances and in these cases the intermediate stage of colloid goitre is not seen. The simple hyperplasia of normalcy is not followed by the normal resting stage nor is the hyperplasia slightly in excess of normal, followed by the colloid goitre, but the hyperplasia continues unarrested. The demand apparently cannot be supplied, but nature is not deterred in her efforts to maintain a metabolic equilibrium and the thyroid gland in its attempt to function in its specialized capacity continues its increased activity with resultant diffuse hyperplasia.

One is then dealing with the second type or hyperthyroidism; the goitre with diffuse hyperplasia in which is not found much evidence of the colloid resting stage, but marked activity of the gland. This type is associated with severe constitutional disturbances. If the patient has been known to have had a goitre previous to the onset of symptoms the clinician applies the term "toxic adenoma;" if the patient gives no history of previous goitre the diagnosis "exophthalmic goitre" or "Graves' disease" is applied. Both of these terms serve as an aid and offer an advantage in establishing a clinical entity, but fail in properly classifying he goitre pathologically. The histological picture is essentially one of cellular proliferation and in the advanced case there is actual papillary formation. True one may find many areas in which

the colloid follicles, the contained colloid and the interfollicular stroma are in normal relationhip; a few areas may even show some evidence of the colloid resting state in which the follicles are dilated and filled to their utmost capacity wih colloid. This is merely further evidence that this condition, although it may suggest a separate entity by its clinical manifestations, is only part of some progressive and continuous process.

Should hyperplasia continue unarrested there is a very rapid process with very marked clinical manifestations. The hyperplasia continues diffusely throughout the gland, with absence of involution and resting stage. The onset and continued growth are both very rapid and there seems to be complete absence of thyroid readjustment such as occurs in arrested hyperplasia and involution; a diffuse hyperplastic goitre commonly called Graves' disease by the clinician is the result. In this particular type probably more than in any other, the histological picture is in agreement with the clinical symptoms. The unarrested continued hyperplasia, evidencing marked cellular activity is manifested clinically by the usual symptoms of a very active goitre. The pathologist, even here however, cannot make an interpretation relative to the symptoms which this marked increased activity may produce. For instance, he cannot say whether or not exophthalmus is one of the clinical symptoms, no matter how marked the hyperplasia is on histological examination; nor can he state the degree of toxicity such a histological picture may produce. The terms "exophthalmic," "toxic" and the like are therefore not the best ones to be used in giving a pathological interpretation. Instead I prefer to report the hyperplasia as the essential feature and by way of further explanation state the degree as slight, moderate or very marked and whether or not it is sufficiently marked to produce papillary infolding.

Occasionally the patient may withstand the bodily abuse by the unarrested hyperplastic thyroid and the process continues until there is cellular exhaustion. Without arrest the cells continue in their active hyperplasia until completely exhausted. The high granular active cell becomes flat and inactive and is finally desquamated and absorbed. This is commonly referred

to as the "burned out" gland; while the patient survives, marked pathological changes result with accompanying symptoms of hypothryoidism having been preceded by hyperthyroidism.

At any stage before exhaustion however in this progressive hyperplastic course a readjustment may assert itself and a resting state results. The gland then assumes a resting state, related to, but only as close to the normal as possible, depending upon the degree of the preceding hyperplasia. If the preceding hyperplasia is only of the mild type which produces no clinical symptoms, the arrested state will result in simple colloid goitre which is usually smooth and diffuse. If the preceding hyperplasia has passed this stage and has produced clinical symptoms the arrested state will result in circumscribed areas of colloid with possible nodular formation as compared to the smooth and diffuse simple colloid goitre. The circumscription is due to increased fibrous connective tissue in the interlobular stroma forming connected strands which completely enclose areas of more rapidly growing colloid containing follicles. These nodular colloid goitres differ from the simple colloid goitres only in that the preceding hyperplasia had been farther progressed before it was arrested. When arrest of the hyperplasia does finally occur the process is probably somewhat farther advanced and more intricate than that which results in the colloid goitre. It is highly probable that the hyperplasia continues at least for a longer period, or grows much more rapidly in certain areas with the production of the nodules at these points. Such a picture may be seen in the thyroid of a patient who showed marked symptoms of hyperthyroidism, and who was given iodine for ten days or two weeks prior to operation. If in such a case iodine is continued over a long period of time a diffuse hyperplastic goitre may be changed to a nodular colloid goitre which still shows areas of hyperplasia. Rienhoff[4] demonstrated such involutionary changes from extreme hyperplasia to the colloid resting state. The natural process of readjustment or arrest of the hyperplasia seems to present a similar picture but is apparently much slower in its course.

The cellular proliferation in the gland with or without papillary infolding is usually associated with the symptoms of toxicity

found in the toxic goitres. In a general way it may be stated that the severity of the toxic symptoms bears a direct proportional relationship to the degree of cellular proliferation. It is a mistake however to assume that the entire gland of a patient presenting symptoms of thyrotoxicosis is made up of proliferating follicular cells. In many cases the larger part of the cut section is made up of small colloid follicles and very little active cellular proliferation. In other cases the larger part of the gland is made up of active cellular proliferation with marked papillary infolding. The varying degree of colloid containing areas in this proliferating type of goitre may bear some relationship to the other types of goitre with moderate colloid containing areas.

In studying a large number of goitres one finds that the dividing line between diffuse hyperplastic or exophthalmic goitres and toxic nodose goitres or the so called toxic adenomata may be only the route the gland has traveled in this process of hyperplasia-involution cycles. Continuous unarrested hyperplasia with little or no evidence of involution results in diffuse hyperplasia of the exophthalmic type. Hyperplasia followed by involution and constant repetition of this process, of some not definitely known stimulation, results in the nodular form of goitre, the toxicity of which may depend upon the degree of suddenly developed hyperplasia which is not counteracted by involution. In many of these cases if a careful history is taken, the toxic type will be found to be an acute exacerbation of an old chronic state rather than a new or distinct process. It seems that greater clarity is achieved by considering this toxic nodular form of goitre as one of the variations in this continuous hyperplasia-involution process instead of separating them from pure colloid or nodular colloid and degenerated goitres. All goitres may therefore be considered as various stages with individual variations of a single thyroid disease. In conformity with this conception the various groups or types of goitre, due to functional disturbances, listed in chart 2 may be looked upon as the various stages in the development of goitre rather than separate types or kinds of goitre.

I must add with regard to adenomata that not all thyroid

nodules or nodular goitres found are of the origin here contended. A small percentage, reported by Rienhoff and Lewis series in 109 cases as 8 per cent, are most likely true neoplasms and deserve the term adenomata. Their character however differs from these encapsulated areas here described. The encapsulation does not make them true tumors, but the internal structure does. Micro-

1. Diffuse goitre
   a. Diffuse simple colloid goitre
   b. Diffuse parenchymatous goitre (some forms of adolescence)
   c. Diffuse hyperplastic goitre (exophthalmic)
   d. Diffuse goitre of myxedema (exhaustion fibrosis)
2. Nodose goitre
   a. Nodose colloid goitre
   b. Nodose goitre with fibrosis
   c. Nodular parenchymatous goitre (adenoma)
   d. Nodose hyperplastic goitre (adenoma or exophthalmic or both)

CHART 2. GOITRE CLASSIFICATION

1. True adenoma
2. Carcinoma
3. Langhan's proliferating goitre
4. Benign metastasizing goitre
5. Sarcoma

CHART 3. TUMORS OF THE THYROID GLAND

| | |
|---|---|
| Primary or secondary inflammation | Thyroiditis (possibility of diffuse hyperplasia-resultant hyperplastic goitre) |
| | Strumitis fibrosis |
| | Syphilis, tuberculosis, etc. |
| | Riedel's strumitis |

CHART 4. INFLAMMATORY LESIONS OF THE THYROID GLAND

scopically they show masses of epithelial cells, forming very early follicles which show no lumen; these cells are in strands which anastomose with each other. The blood vessels are quite abundant but no where is there evidence of open hemorrhage. The hyperplasia-involution process is not seen at any stage, nor is there any evidence of lone hyperplasia. These are true ade-

nomata definitely neoplastic in character, and therefore of entirely different origin than the various types of goitre developed through the pathogenesis here described. (See chart 3.)

While infection of the thyroid gland may bear some relationship to the disturbances explained on a basis of dysfunction, we prefer to consider inflammatory disturbances of the gland in a separate class and may classify them as in chart 4.

## CONCLUSIONS

In the light of present goitre etiology, I conclude that these colloid nodular goitres or so called colloid adenomata are the result of degeneration dependent upon hyperplasia followed by involution and hyper-involution. That the so called toxic adenomata differ only from the non-toxic colloid nodular goitre in that they contain areas of hypo-involution, in which hyperplasia was not followed by involution. That the so called toxic adenomata are frequently not toxic per se, in which case they are pathologically the same as the non-toxic type, but are associated with hyperplasia in a separate portion of the thyroid not included in the encapsulated nodule.

Further it is highly probable that similar factors are involved in the production of both hyperplastic and colloid types of goitre. That the simple colloid goitre is only an end result of hyperplasia which is mild and not rapid in its course. That the nodular colloid goitre is of similar origin, but differs in that the hyperplasia-involution cycle occurred only in certain areas. That the toxic nodular colloid goitre or toxic adenoma is also of the same origin, but here while the hyperplasia-involution cycle occurs there are a few areas of hypo-involution in which hyperplasia persists. That the true hyperplastic or exophthalmic goitre is one in which there is marked very rapid and unarrested hyperplasia not followed by any form of involution.

## REFERENCES

(1) GRAHAM, ALLEN: Nodular goitres: their relation to neoplasia. Am. Jour. Surg., **7:** 163–173. 1929.

(2) HERTZLER, A. E.: Pathogenesis of goitre considered as one continuous disease process. Arch. Surg., **16:** 61–78. 1928.

(3) MARINE, D.: The importance of our knowledge of thyroid physiology in the control of thyroid disease. Arch. Int. Med., **32:** 811–827. 1923.

(4) RIENHOFF, WM. F., JR.: Involutional or regressive changes in the thyroid gland in cases of exophthalmic goitre. Arch. Surg., **13:** 391–425. 1926.

(5) RIENHOFF, WM. F., JR., AND LEWIS, DEAN: Relation of hyperthyroidisim to benign tumors of the thyroid gland. Arch. Surg., **16:** 79–116. 1928.

# ANTIRABIC VACCINATIONS AT THE CHARITY HOSPITAL OF NEW ORLEANS FOR THE YEARS 1929–1930

RIGNEY D'AUNOY AND J. L. BEVEN

*From the Departments of Pathology of the Charity Hospital, and the School of Medicine of Louisiana State University, New Orleans, Louisiana*

During 1929 and 1930, the Pasteur Institute of the Charity Hospital administered antirabic prophylactic treatment with material prepared as generally indicated by Semple. We desire to present a tabulation of the treated cases, as well as certain statistical considerations thereon and to record briefly methods of vaccine production and to make some observations on post vaccinal results.

## PRODUCTION OF VIRUS

All of the vaccine-material used was produced with a strain of fixed rabic virus secured through the kindness of Parke, Davis and Company. Subdural injection of full grown rabbits with emulsions of infected brain stems or cords were accomplished in the usual manner. With the advent of complete paresis, usually on the seventh day after inoculation, the animals were killed by ether narcosis in order to avoid the so common agonal bacterial invasion of the central nervous system noted when such infected animals are allowed to die. The dead animals were immersed in 5 per cent Lysol solution for five minutes, skinned, and again dipped in fresh 5 per cent Lysol solution. After light external flaming, the cord and brain were removed under strict aseptic conditions. Cultures from internal and external portions of the brain and cord were made in 0.05 per cent dextrose broth contained in fermentation tubes. After securing the cultures, an 8 per cent emulsion of brain-cord in normal saline solution was prepared, filtered through three layers of fine linen and 1 per cent carbolic acid added. The carbolized emulsion was incubated at

37.5° for twenty-four hours, after which time it was diluted to 4 per cent by the addition of sterile normal salt solution. Of this emulsion 0.5 mil portions were injected subdurally into each of two full grown rabbits and fifteen glucose broth cultures in five series of five, ten and twenty drops were made. Subsequent similar sub-cultures were made from these original cultures after they had been incubated for five days at 37.5°. If the test animals were living and well three weeks after injection, and if not more than one of each cord-brain culture, and not more than one emulsion culture in any series showed contamination with any type of organism, the material was considered ready for use and a dating of three months from date of carbolisation allowed.

## METHOD OF TREATMENT

The following plan of treatment was used, all injections consisted of 2 mil portions of 4 per cent killed virus-emulsion, except for children less than three years of age in whom 1 mil portions of vaccine were injected at a treatment.

### *Injuries by proven rabid animals*

*Head injuries.* Injections were made twice daily for the first seven days and once daily thereafter for 14 days.

*Injuries to trunk and extremities.* If multiple and severe, the same treatment was used as for head injuries.

If slight and treatment was begun within six days after injury, treatments were continued for fifteen days, one injection daily.

If slight and treatment was begun more than six days after injury, treatments were continued for eighteen days, one injection daily.

### *Injuries by unlocated animals*

With no suspicious circumstances, fourteen days treatment one injection daily.

With suspicious circumstances, same treatment as for similar type of injury by proven rabid animals.

### *No actual injury*

If rabid or suspicious animals had been handled, eighteen days treatment was given with one injection daily.

## TREATED CASES

There were 766 patients treated during 1929, and 467 during 1930. They were classified in the following categories as suggested by the International Rabies Conference of the League of

TABLE 1

CLASSIFICATION BY LOCATION OF INJURY OF 1233 PATIENTS (766 IN 1929, 467 IN 1930) TREATED PROPHYLACTICLY AGAINST RABIES

| LOCATION OF INJURY | 1929 | | | | | 1930 | | | | | 1929–1930 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Category | | | | | Category | | | | | Category | | | | |
| | A | B | C | D | E | A | B | C | D | E | A | B | C | D | E |
| Head | 38* | 2 | 25 | 7 | | 5 | | 9 | 7 | | 43* | 2 | 34 | 14 | |
| Body | 23 | | 7 | 1 | | 2 | 2 | 5 | 2 | | 25 | 2 | 12 | 3 | |
| Superior extremities | 161 | 9 | 80 | 17 | | 79 | 1 | 40 | 14 | | 240 | 10 | 120 | 31 | |
| Inferior extremities | 133 | 6 | 54 | 12 | | 44 | | 51 | 11 | | 177 | 6 | 105 | 23 | |
| Multiple sites | 11 | 1 | 6 | | | 19 | 1 | 16 | 11 | | 30 | 2 | 22 | 11 | |
| Totals | 366* | 18 | 172 | 37 | 172 | 149 | 4 | 121 | 45 | 148 | 515* | 22 | 293 | 82 | 320 |

* One other patient in this category was treated but died.

TABLE 2

AGE CLASSIFICATION OF PATIENTS TREATED

| AGE | 1929 | | | | | 1930 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | White | | Colored | | Total | White | | Colored | | Total |
| | Male | Female | Male | Female | | Male | Female | Male | Female | |
| *years* | | | | | | | | | | |
| Under 1 | 0 | 1 | 0 | 0 | 1 | 2 | 2 | 0 | 0 | 4 |
| 1–2 | 21 | 17 | 3 | 1 | 42 | 15 | 5 | 2 | 2 | 24 |
| 3–4 | 54 | 22 | 5 | 4 | 85 | 16 | 15 | 3 | 3 | 37 |
| 5–9 | 108 | 63 | 15 | 11 | 197 | 53 | 49 | 9 | 4 | 115 |
| 10–19 | 86 | 45 | 10 | 4 | 145 | 42 | 40 | 10 | 5 | 97 |
| 20–29 | 42 | 33 | 9 | 12 | 96 | 22 | 26 | 6 | 4 | 58 |
| 30–39 | 34 | 51 | 1 | 4 | 90 | 26 | 29 | 5 | 4 | 64 |
| 40–49 | 26 | 22 | 1 | 5 | 54 | 16 | 12 | 3 | 4 | 35 |
| 50–59 | 16 | 18 | 2 | 1 | 37 | 11 | 9 | 2 | 0 | 22 |
| 60–69 | 6 | 4 | 1 | 1 | 12 | 3 | 4 | 1 | 1 | 9 |
| 70–79 | 2 | 3 | 1 | 1 | 7 | 0 | 1 | 0 | 0 | 1 |
| Over 80 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | 395 | 279 | 48 | 44 | 766 | 206 | 193 | 41 | 27 | 467 |

Nations in order that the statistics of various institutions may be comparable:

A. Animals proved rabid (microscopic or biologic test).
B. Animals diagnosed as clinically rabid.
C. Animals only suspected (stray, destroyed, or in such a state when received that the brains were unfit for examination).
D. Animals alive and well after an observation period of three weeks, or with negative brains examined after the observation period.
E. Cases undergoing treatment without actually having been bitten (handling positive or suspected rabid animals).

TABLE 3

GEOGRAPHICAL DISTRIBUTION OF PATIENTS TREATED

| PARISHES | 1929 | 1930 |
|---|---|---|
| Allen | 2 | 0 |
| Assumption | 2 | 21 |
| Ascension | 15 | 3 |
| Avoyelles | 7 | 1 |
| Concordia | 1 | 0 |
| Evangeline | 2 | 0 |
| Iberia | 1 | 0 |
| Iberville | 1 | 0 |
| Jefferson | 45 | 50 |
| La Fourche | 1 | 3 |
| Orleans | 598 | 378 |
| Plaquemine | 1 | 0 |
| Pointe Coupee | 9 | 2 |
| St. Bernard | 17 | 0 |
| St. Charles | 16 | 1 |
| St. Helena | 3 | 0 |
| St. James | 9 | 0 |
| St. John the Baptiste | 23 | 1 |
| St. Tammany | 3 | 6 |
| Tangiopahoa | 4 | 0 |
| Terrebonne | 2 | 1 |
| Washington | 2 | 0 |
| W. Baton Rouge | 2 | 0 |
| | 766 | 467 |

Table 1 records the treated cases in each category together with the types of injury and death. Table 2 indicates the age of treated cases; table 3, their geographical distribution in Louisiana.

TABLE 4

TIME ELAPSING BETWEEN INJURY OR EXPOSURE AND BEGINNING TREATMENT

| 1929 | | 1930 | |
|---|---|---|---|
| Days | Patients | Days | Patients |
| 1 | 215 | Same | 8 |
| 2 | 92 | 1 | 88 |
| 3 | 79 | 2 | 69 |
| 4 | 56 | 3 | 54 |
| 5 | 53 | 4 | 36 |
| 6 | 58 | 5 | 20 |
| 7 | 44 | 6 | 20 |
| 8 | 48 | 7 | 39 |
| 9 | 23 | 8 | 24 |
| 10 | 24 | 9 | 24 |
| 11 | 14 | 10 | 11 |
| 12 | 7 | 11 | 9 |
| 13 | 12 | 12 | 18 |
| 14 | 11 | 13 | 7 |
| 16 | 2 | 14 | 9 |
| 18 | 2 | 15 | 5 |
| 19 | 1 | 16 | 5 |
| 20 | 1 | 17 | 5 |
| 21 | 5 | 18 | 1 |
| 22 | 6 | 22 | 1 |
| 23 | 4 | 25 | 1 |
| 24 | 2 | 27 | 10 |
| 25 | 1 | 28 | 1 |
| 30 | 3 | 30 | 1 |
| 31 | 1 | 45 | 1 |
| 34 | 1 | | |
| 53 | 1 | | |

TABLE 5

CIRCUMSTANCES OF INJURY AND LOCAL TREATMENT

| | 1929 | 1930 |
|---|---|---|
| Injury inflicted through clothing | 189 | 118 |
| Injury inflicted to bare skin | 374 | 201 |
| Tetanus anti-toxin, nitric acid cauterization to site of injury | 386 | 203 |
| Iodine applied to site of injury | 43 | 14 |
| No local treatment | 134 | 102 |

Table 4 gives the time elapsing before injury or exposure and beginning of treatment. Table 5 indicates the number of injured patients that received local treatment before antirabic vaccination and whether injury was inflicted through the clothing or to the bare skin.

### POST VACCINAL RESULTS AND COMPLICATIONS

One patient, seven years old, died of rabies forty-one days after completion of treatment. He had been severely injured about the face by a proven rabid dog and treatment was begun fourteen hours after injury. No post vaccinal paralysis was encountered.

Local reactions at the site of inoculation, usually beginning after the sixth or seventh injection and appearing with from one to ten injections thereafter were noted in 8 per cent of cases. In two cases a disagreeable, generalized, urticarial-like rash, persisting for two or three days and coming on early during treatment, was noted.

# DEGENERATIVE LESIONS OF THE APPENDIX (APPENDICOSIS) HITHERTO UNDIFFERENTIATED FROM APPENDICITIS

BERNHARD STEINBERG

*From the Laboratories and the Department for Medical Research, Toledo Hospital, Toledo, Ohio*

During the routine examination of appendices removed at operation certain abnormal changes were observed in some which ordinarily are classified by the pathologist as normal although the clinician observed in the patient, signs and symptoms which were relieved by appendectomy. These observations prompted a systematic study of 1500 surgically removed appendices. The study included the correlation in a selected number of cases of the clinical history, the pathologic findings, the bacteriological examination of the appendicial contents and a follow up of the patients' condition a year or more after the appendectomy. The present report concerns itself with the conclusions derived from the correlations between the clinical manifestations and the pathologic changes.

## PATHOLOGY OF RETROGRESSIVE AND DEGENERATIVE LESIONS OF THE APPENDIX

As a result of this study, a group of appendices in which inflammation played no part in the pathological picture was differentiated. The abnormal changes were retrogressive and degenerative in character and terminated in death of tissue. The term appendicosis is proposed to designate the pathologic changes in these appendices. Two types of appendicosis were distinguished. The one essentially retrogressive in nature is characterized by a diminished width of the mucosal layer of the organ, a compression of lymph follicles, a general decrease in the number of mucosal cells and an increase in fibroblasts. Frequently, the usual lining columnar cells are replaced either by cuboidal or flat epithelial

cells. The muscularis may participate in the general diminution of the width of the organ. The lumen of the organ is dilated and almost invariably contains large amounts of fecal material. Occasionally, proximal to the dilated part of the organ there is narrowing or complete stenosis of the lumen. For this type of appendicosis the term pressure atrophy is proposed. It is the belief of the writer that the pressure atrophy type of appendicosis is due to the failure of the appendix to properly expel the fecal

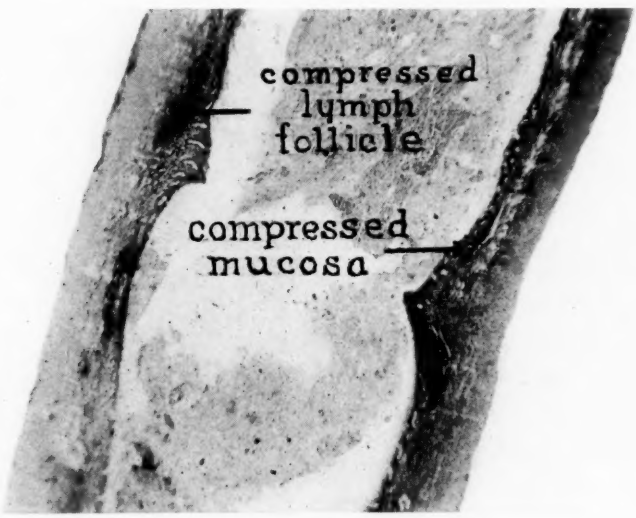


FIG. 1. APPENDICOSIS, PRESSURE ATROPHY TYPE (NO. 32 MICROTESSAR)

The lumen is dilated. The mucosa is compressed. The lymph follicles are likewise compressed and there is a general diminution of the mucosal cellular content.

contents which continue to accumulate and result eventually in the compression of the wall of the organ. (See fig. 1.)

The second type of appendicosis is degenerative in character and is distinguished by the tendency to formation of erosions. A small area of the lining cells and the mucosa is destroyed and replaced either by blood in lesions of recent origin and by feces or by connective tissue in lesions of longer duration. These erosions frequently extend through the entire width of the

mucosa to the submucosa. The erosions may be single or multiple. In comparatively old lesions the eroded area is surrounded by free and phagocytosed hemoglobin particles. Not infrequently, the same appendix may show, in other places, evidences of pressure atrophy. The lymph follicles are usually hyperplastic with large and active germinal centers especially in early lesions. There is

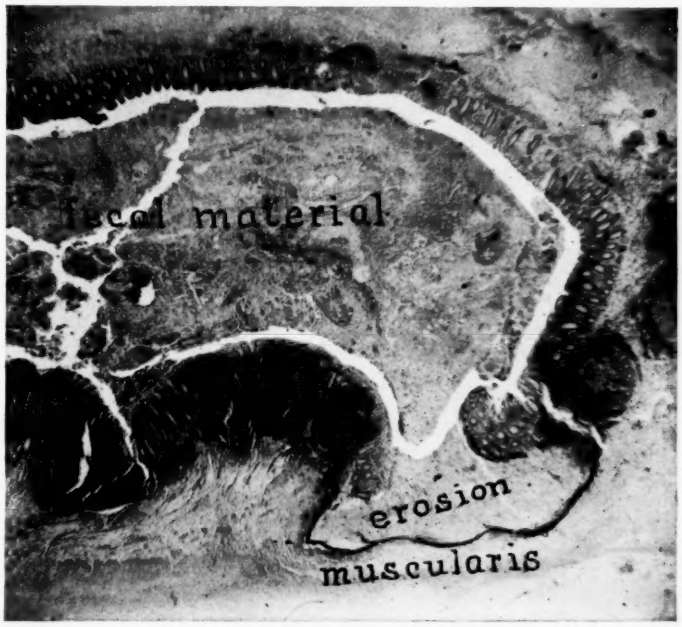


FIG. 2. APPENDICOSIS, MUCOSAL EROSION TYPE (NO. 32 MICROTESSAR)

In one place there is destruction of the greater part of mucosa and the defect is filled with material which under higher magnification is demonstrable as blood. The mucosa on either side of the defect contains hyperplastic lymph follicles.

frequently an increase of eosinophiles and large mononuclears in the mucosa surrounding the erosion. The tubular glands show moderate secretory activity. The lumen in the location of the eroded area may be very narrow but is occasionally wide. The erosion is most frequently located at the base of a crypt. Erosion type of appendicosis is the term proposed for this form of

appendicial lesion. It is my opinion that the erosion type of appendicosis is produced by sharp particles of feces and the lack of an inflammatory reaction is due either to absence of bacteria or presence of avirulent organisms. (See figs. 2 and 3.)

### PATHOLOGY OF INFLAMMATORY LESIONS OF THE APPENDIX

The etiology of appendicitis has been a topic of controversy and speculation since the days of Melier (1827), Fitz (1886) and

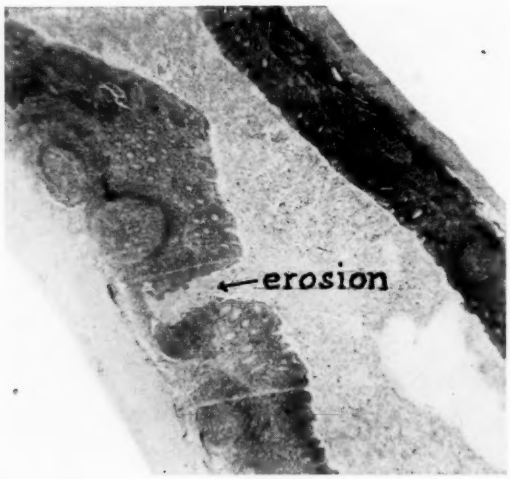


FIG. 3. APPENDICOSIS, MUCOSAL EROSION TYPE (NO. 32 MICROTESSAR)

There is destruction of the entire mucosa and submucosa in one area. The base of the defect consists of heavily stained connective tissue and the defect is filled with fecal material. The mucosa on each side of the erosion shows hyperplasia of the lymph follicles as contrasted with the follicles of the mucosa on the opposite side of the erosion.

Fowler (1894) and remains in the same status today. There are two schools of thought regarding the cause of appendicitis. One is sponsored by Rosenow[2] who is of the belief that bacteria are carried by the blood to appendicial blood vessels where they lodge and set up an inflammation. The cause of the other school is espoused among others by Warren[3] who finds no justification in the belief of the hemotogenous embolic etiology of appendicitis.

It is the general consensus of opinion that the disease is produced ordinarily from an injury to the mucosa and a consequent invasion of virulent bacteria. The hemotogenous embolic route, if a fact, is of rare occurrence. Observations made in this study apparently bear out the conception of mucosal injury and bacterial invasion from the lumen of the organ.

The initial type of lesion noticed in this study was a small ulceration of the mucosa. The lining cells were destroyed and a small area of the mucosa infiltrated by polymorphonuclears. The ulceration may extend through part of the mucosa or to the submucosa. The ulcer may be single without presence of any further evidences of inflammation elsewhere in the organ. This was verified by an examination of longitudinal sections of the entire appendix. Occasionally, the ulcers were multiple. It is assumed by the writer that the mucosal ulcer constitutes the site of the primary injury. The ulcer may heal with consequent fibrosis or the inflammation may extend to the entire mucosa or the whole wall of the appendix. The term appendicitis as introduced by Reginald Fitz admirably fits this type of appendicial disease. For a clearer pathological differentiation I am proposing the following types of appendicitis: (1) mucosal ulcer type in which single or multiple ulcerations of part or the whole width of the mucosa are present, (2) mucosal appendicitis, consisting of a diffuse polymorphonuclear infiltration of the entire mucosa with or without the presence of demonstrable ulcers, (3) diffuse appendicitis in which the entire appendicial wall is infiltrated by polymorphonuclears and other inflammatory cells, (4) gangrenous appendicitis, with or without perforation, where in addition to the diffuse appendicial lesion, there are areas of necrosis which if extending through the entire width of the organ constitute a perforation.

### APPENDICIAL CONCRETIONS AND FIBROSIS

Appendicial concretions commonly referred to as fecaliths have been described by many observers. The composition of these concretions has been a subject of much controversy. It is generally accepted at the present that the concretions consist principally of hard fecal material with an occasional central core of some

foreign body. When concretions are found in the lumen of the appendix following an appendicial attack the mucosa usually shows areas of hemorrhage. The lining epithelium in the location of the concretion may be cuboidal or flat in type. There is not infrequently free blood in the lumen. Associated with the concretion may be found any type of appendicosis or appendicitis. Dilatation of the lumen with presence of blood in the mucosa without a mucosal erosion usually indicates that a concretion has been present but was expelled. The term appendolithiasis is proposed for those conditions in which a firm mass of any composition is found in the lumen of the appendix.

There are two views regarding fibrosis of the appendix. One holds that the fibrosis is a senile change, the other that an inflammatory reaction always precedes fibrosis. From this study, it is the view of the author that fibrosis of the appendix follows most frequently the mucosal erosion type of appendicosis and less frequently the mucosal ulcer type of appendicitis. It is not within the scope of this paper to enter into various reasons for this view. The fibrosis observed was either focal or diffuse in type. The focal type is frequently present at the tip of the organ but may involve any part of the appendix with connective tissue replacement of the mucosa and lumen. Occasionally there is stenosis of a part of the lumen with dilatation of the distal or proximal portions. In the diffuse type, the entire appendicial mucosa and lumen are replaced by connective tissue. The terms appendicial fibrosis, focal and diffuse types are proposed for these conditions.

*Pathologic classification of degenerative and inflammatory diseases of the appendix*

1. Appendicosis
   a. Mucosal erosion type.
   b. Pressure atrophy type.
2. Appendicitis
   a. Mocosal ulcer type.
   b. Mucosal appendicitis.
   c. Diffuse appendicitis.
   d. Gangrenous appendicitis with or without perforation.
3. Appendolithiasis
4. Apendicial fibrosis
   a. Focal type.
   b. Diffuse type.

## CLINICAL MANIFESTATIONS OF APPENDICOSIS*

With the pathological classification established, a number of clinical histories were distributed under each of the classifications for correlation. It was found that no clinical differentiation could be made between the mucosal erosion and pressure atrophy types, only the diagnosis of appendicosis could be established. Appendicosis occurred as acute or intermittent attacks extending over weeks, months, or years. One group had continuous symptoms over long periods. The clinician's diagnosis of the cases showing these prolonged clinical manifestations was that of chronic appendicitis. There has been considerable controversy regarding this term. Pathologists are practically in unanimity in denying the presence of such a condition. Aschoff[1] explained the presence of clinical manifestations over long periods by repeated attacks of appendicitis. However, this study reveals that recurrent attacks of an inflammatory disease of the appendix (appendicitis) is not common and that the chronic appendicitis of the clinician is appendicosis of either type or the combined types.

In appendicosis pain was usually dull in character. In patients who had attacks of sharp pain, appendicial concretions were found. There was sometimes slight nausea during the attack. Vomiting occurred very infrequently. There was usually slight to moderate tenderness on deep palpation over the right iliac fossa but rigidity and muscle spasm were absent. There was no fever, the temperature never being found over 99.2°F. The total number of peripheral leucocytes was usually between 8,000 and 9,000 and only occasionally rose to between 10,000 and 11,000. If the appendicosis was intermittent or continuous, patients complained of a more or less continuous abdominal discomfort. There was frequent constipation, lassitude and an indefinite general indisposition.

The clinical picture of a true inflammation of the appendix is so clear cut in the mind of the clinician that it is unnecessary to reiterate the various manifestations. From this study it is the

* I am greatly indebted to Dr. W. M. Stevenson for compiling many of the clinical histories.

belief of the author that one cannot make a distinct clinical differentiation of mucosal ulcer, mucosal or diffuse appendicitis. The clinical manifestations of appendicitis are presented here only as a contrast to those of appendicosis. In appendicitis the pain is usually colicky, or sharp in the presence of a concretion. There is nausea and vomiting. The abdomen over the right iliac fossa is tender to pressure and there is rigidity and spasm. The temperature is slightly elevated and the peripheral total white count is increased, varying between 12,000 and 40,000. In mucosal ulcer types, the symptoms and signs are generally milder in character but mucosal or diffuse appendicitis may simulate the former type. Much has been written in regard to recognition of gangrenous appendicitis with perforation and the author has little to add to this phase of the subject.

*Clinical classification of degenerative and inflammatory diseases of appendix*

1. Appendicosis
2. Appendicitis
   a. Mucosal ulcer type (questionable of clinical diagnosis).
   b. Diffuse type.
   c. Gangrenous with perforation.
3. Appendolithiasis (clinical diagnosis presumptive only)

SUMMARY

A retrogressive and degenerative lesion of the appendix is differentiated pathologically and clinically. A set of signs and symptoms which had been included under the term appendicitis are differentiated and are ascribed to a pathological entity which is termed appendicosis. A new pathological and clinical classification of the inflammatory and degenerative diseases of the appendix is proposed.

REFERENCES

(1) Aschoff, Ludwig: Über Chronische Appendicitis. Med. Klin., **24:** 1660. 1928.

(2) Rosenow, E. C.: The bacteriology of appendicitis and its production by intravenous injection of streptococci and colon bacilli. Jour. Inf. Dis., **16:** 240–268. 1915.

(3) Warren, Shields: Etiology of acute appendicitis. Amer. Jour. Path., **1:** 241–246. 1925.

## EDITORIAL

### Which Test for Syphilis?

For a quarter of a century the Wassermann reaction has held sway as the most valuable single laboratory procedure for the diagnosis of syphilis in all of its many phases. That it is still of value and the standard by which other tests are judged none will gainsay. However, the many modifications, and the hundreds of "comparative studies" that have been reported have long since shown the nonspecific nature of the reaction, and the fallacy of pinning one's faith to any one technic. In the last few years interesting and favorable reports have been received on various precipitation tests, or preferably, flocculation tests, as the aggregated material that becomes visible in a positive test is not a precipitate from a true solution. From Europe has come the technic of Sachs-Georgi, Vernes, and Meinecke. But investigators in this country are more interested in the Kline, Kahn and Hinton tests. These methods are rapidly gaining in favor also in Europe. The advocates of each test have written voluminously of their comparisons with other methods, all lauding their favorite and basing their conclusions on percentages of agreement, which vary from 70 to 95 per cent, depending on which and how many tests are compared. Let it be admitted that these are all good tests, but that none is infallible. Why not use them all? With 13 cc. of blood, the Kline, Kahn, Hinton and Kolmer tests can all be performed, and the comparison of the results will be of value to the syphilologist, often in those instances in which there is lack of agreement in the results.

The following routine is very useful in effecting a saving both in time and materials. For the preliminary test, before a diagnosis has been made, only 3 cc. of blood are drawn. A Kline test is done at once, using the very sensitive antigen. A negative result with this test, if there is not a history of syphilis, can be taken as

sufficiently final, and further tests need not be done. If the Kline test is positive in any degree, a Kahn test can then be done for confirmation. If this is negative, the clinician must decide whether he wishes further investigation. If the history suggests syphilis, or if both of the rapid flocculation tests are positive, 13 cc. of blood are taken for more complete serologic examination. The very sensitive micromethod of Kline and the Kahn test are again done, and a Hinton test is also set up. The last-named test requires careful technic, and overnight incubation in an accurately controlled water-bath. The reactions with this test are all that are claimed by the originator: they are easily read, are highly accurate, and are persistently positive after treatment and in cases of cerebrospinal syphilis, when other tests on the blood have become negative. Then the old standby, the Kolmer modification, is also set up. The results of all four of these tests are then reported the next day, and the clinician finds his confirmation of his general examination in the agreement, or even in the disagreement, of the results.

A. H. Sanford.

## NEWS AND NOTICES

Clinical pathologists will be interested in an article by W. J. Bell, Deputy Minister of Health of Ontario, on the State's relation to the practice of medicine, which appeared in the Texas State Journal of Medicine, July, 1930. Dr. Bell takes issue with anyone who suggests that preventive medicine is a function of the State and therapeutic medicine is a function of the private physician, claiming that no definite line of demarcation can be properly drawn. State medicine, in his opinion, should be based on whether the whole community is involved in the solution of the medical problem. He admits that for purposes of propaganda the State may well introduce a measure for public health into a community but after that the matter should rest with the private physician.

Two groups of patients, however, must be cared for by the State: (1) Those who are unable to afford medical treatment and (2) those pioneers in a sparsely settled area. However, the medical care of these groups should not slip from the control of the organized medical group and the State should employ the medical profession either individually or collectively to render this service. After setting up these rules, Dr. Bell things that the laboratory branch of medicine should be operated by the State and that for the present the outstanding need is more accurate and modern facilities for diagnosis throughout the medical profession. He states "it is not to be expected that the practicing physician, especially the busy general practitioner, will be conversant with the technique employed in these various laboratory procedures and will be able to perform the tests." He suggests that the State provide at suitable locations a staff for a clinical laboratory center and sufficient number of fully qualified physicians, technically trained and with suitable assistants, and that the initial cost of equipment and housing should be borne by the local community and that the annual maintenance charges

be borne by the central Government and that a minimal charge be made for laboratory services. Service should be rendered only upon written request signed by a physician and reports should be delivered to him, the laboratory in no way assuming direct responsibility for the patient.

The effect of such a scheme is obviously to place the State in competition with private laboratories and even to do away with such laboratories. It would to some extent reduce the need for laboratories in hospitals, although Dr. Bell would carefully guard the interests of the private practicing physician or surgeon. Many clinical pathologists will see in this plan a serious menace to the development of the clinical laboratory, yet the definiteness of the scheme and the fact that to some degree it already exists will warrant its thorough discussion.

Announcement has just been received of the opening, October 1931, of Louisiana State University's Medical Center in New Orleans, to be domiciled in its own quarters. Included in the new Medical Center is Charity Hospital.

Attention of Clinical Pathologists is directed to a letter and blank form, "Survey of Laboratories," which was sent out by the Secretary of the Council on Medical Education and Hospitals of the American Medical Association, under date of May 22.

This communication indicates that the list of pathologists conducting approved clinical laboratory service which was heretofore limited to those who were conducting independent laboratories, is now to be extended to all physicians specializing in clinical pathology and conducting clinical pathology laboratory service whether connected with a hospital or not. Attention is directed to the fact that the American Medical Association has published in a recent issue of the Journal the essentials of an approved clinical laboratory, yet the list of such laboratories as published is a list of pathologists and not of laboratories, the inference being that those pathologists are conducting approved laboratories. One finds, however, that an approved laboratory must be prepared to render service in five different and rather widely separate fields of medicine.

It is quite conceivable that a pathologist might meet with the whole-hearted approval of any examining board, but that he might not be conducting a laboratory which is prepared to give service in more than one or two of these general fields. This usually is true in large institutions where the very nature of the work demands that several men be employed to render adequate service in all of the branches of laboratory work. The laboratories therefore taken as a whole may be quite satisfactory or even superior, but under the manner of listing by the American Medical Association would not properly be listed.

For this reason it is suggested that instead of printing a list of pathologists, a list of approved laboratories he published and the names of those in charge of these laboratories be listed after that of the laboratory. In this way it will be possible to list as a unit the laboratories of a hospital or large institution which may represent the combined group of laboratories being supervised by a number of different pathologists.

It would seem that this would be the only safe way of proceeding, else the American Medical Association will find itself in the position of classifying pathologists as to their relative professional standing.

It is suggested that an expression of opinion on this subject be made directly to the Secretary of the Council on Medical Education and Hospitals.

## BOOK REVIEWS

*Clinical Diagnosis by Laboratory Methods.* Seventh edition. By J. C. Todd and A. H. Sanford. Pp. 765. 1931. Philadelphia, W. B. Saunders Co. $6.00.

Many new features have been added to the revised seventh edition of this well known book. The greater number of references to the literature will serve as an incentive to the medical student to brouse around and to study more deeply the problems which are of special interest to him. This wider reading often correlates the experimental work with the practical, opening new avenues of interest and giving to the student the fundamental principles of the laboratory test as well as the actual procedure, making the test become something more than routine.

A careful reading of the text indicates that this edition includes many improved methods in all branches of laboratory diagnosis, especially in blood chemistry and bacteriology. The electrolytic Gutzeit test for detecting arsenic and Fairhall's method for lead in the urine are two additions to the subject of urinalysis. Hematology is enriched by including a more elaborate discussion of carbon monoxide hemoglobin giving the technic of Katayama's test, and a brief description of new methods for estimating the hemoglobin content of the blood. A modified method for determining volume index is also added. The discussion of isohemagglutination, including subgroups, diagrams, and the bearing on medical legal cases is more detailed than in earlier editions.

The chapter on chemical examination of the blood contains the greatest number of important changes and additions in the text. A reference to Sander's work on preservation of blood for chemical analysis and the formula for preparing the preservative is an aid to the chemist. The grouping of the tests for non-protein nitrogen, urea, creatinin, and uric acid is more logical and less confusing than that found in earlier editions, and several new methods have been added. There is an interesting reference to capillary microscopy with photographs of capillaries in Raynaud's disease. The Aschheim-Zondek test for pregnancy and the Friedman modification are valuable in view of the importance and reliability of the test.

A. S. Giordano.

*Protozoan Parasitism of the Alimentary Tract.* By KENNETH M. LYNCH. Pp. xviii + 258. 1930. New York, The Macmillan Co. $3.75.

The author clearly points out in the introduction that the book is intended for the medical man who is not too concerned about the details of protozoology but has to care for sick people who harbor one or more protozoan parasite, and for the protozoologist who seeks a connecting link between his science and medicine. It is not intended that the biologist or the protozoologist will use it as a reference book.

In the fulfillment of his purpose the author has succeeded, for the book is a running account of the pathology, diagnosis and treatment of intestinal protozoa and deals only superficially with the details of morphology, life history and biology of the various species.

For the identification of amoebas the author insists on the study of cysts after pointing out that *E. histolytica* has four nuclei and *E. coli* eight. He states, "this simple mathematical proposition, so difficult to get across to medical laboratorians, is the most important thing in the diagnosis of amoebiasis, it is the sole conclusive point in the chronic case, practically considered."

This is a statement with which many will disagree and on perfectly sound ground. The *structure* of the nucleus is far more important than the number of daughter nuclei and therefore if cysts are to be used for identification, nothing short of iron-hematoxylin staining is final.

On the whole the author's views on the rôle of protozoa in the cause of disease are conservative and he does not agree with certain enthusiasts who are all too eager to place upon the delicate shoulders of any protozoan the cause of any symptoms or illness of a given patient. His discussions on treatment are also conservative but summarize the best knowledge on the subject.

The book shows careful topography, the illustrations are certainly excellent and it is recommended to all interested or concerned with these protozoan parasites, for the frank discussion of the subject by Dr. Lynch will do everybody good even if the reader does not agree with all the author's conclusions.